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Pancreatic acinar atrophy in German shepherd dogs and rough-coated Collies

Etiopathogenesis and response to long-term enzyme replacement treatment

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Academic dissertation

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1. ABSTRACT

In pancreatic acinar atrophy (PAA) a selective destruction of digestive enzyme-producing acinar cells leads to maldigestion signs typical of exocrine pancreatic insufficiency (EPI). Etiopathogenesis of PAA has remained obscure. A genetic predisposition to PAA has been reported in German shepherd dogs and rough-coated Collies. This study was set out to examine the etiopathogenesis of PAA; to find a method for the early diagnosis of exocrine pancreatic dysfunction, and thus, to study morphological and immunological findings when the disease is in progress. Also, an assessment was made for the long-term prognosis of enzyme replacement therapy in dogs with clinical disease.

Abnormally low serum trypsin-like immunoreactivity, cTLI, values (< 2.5 µg/L; normal range > 5.0 –35.0 µg/L) associated with typical maldigestion signs of EPI, are considered diagnostic for clinical EPI and found in dogs with end-stage PAA. Study I showed that serum cTLI measurement is also valuable for diagnosing subclinical exocrine pancreatic dysfunction. Repeatedly low serum cTLI values (< 5.0 µg/L) were found in 12 German shepherd dogs and rough-coated Collies with no clinical signs of EPI. Laparotomy verified partial pancreatic acinar atrophy (partial PAA) in all the dogs. Pancreatic findings were comparable to those of end-stage PAA, but less severe. Based on this result, it was concluded that repeatedly low serum cTLI values (< 5.0 µg/L) in clinically normal dogs indicate subclinical EPI, and suggest partial PAA.

An ability to diagnose PAA before the development of total atrophy offered an opportunity to study histological and ultrastructural findings whilst tissue destruction was still in progress, in the subclinical phase of the atrophy process (Study II). The main histological finding in the subclinical phase was marked lymphocytic infiltration into the exocrine pancreas. This differed distinctly from the usually mild inflammation detected during end-stage PAA. The inflammatory reaction was most extensive in the border zone areas of normal and affected parenchyma, and the lymphocytes had infiltrated into apparently normal acinar tissue. In areas of more advanced tissue destruction, the findings were similar to those in end-stage PAA. The ultrastructural findings were in accordance with those of the histological study. Based on these findings, the progression of acinar atrophy was divided into lymphocytic pancreatitis with active destruction of acinar structures and the end-stage PAA**.** Thus, the term “atrophic lymphocytic pancreatitis” is preferred to describe the pathological findings.

The role of cellular immunity in the pathogenesis of acinar atrophy was studied by immunophenotyping the lymphocytes infiltrating the tissue (Studies II and III). In the subclinical phase, the majority of the lymphocytes in the exocrine pancreas were T-cells with an almost equal number of CD4+ T-helper cells and CD8+ cytotoxic T-lymphocytes. The CD8+ cells had infiltrated both the affected and the normal parenchyma. They were considered to be the predominant cells in the areas of gradual destruction of the acinar tissue. Screening the humoral immune status included an assessment of serum autoantibodies (Study III). Serum autoantibodies reacting with pancreatic acinar cells were found in some dogs with subclinical and clinical phase of PAA. Although pancreatic-antibodies were not found in the healthy control dogs, the intensity of the positive reaction was so weak that these antibodies cannot be considered pathognomonic or diagnostic for the disease.

This etiopathogenetic study suggests that pancreatic acinar atrophy in German shepherd dogs and rough-coated Collies is the result of autoimmune-mediated atrophic lymphocytic pancreatitis. Both the previous information on the inheritance of PAA and the current findings of marked T-lymphocytic inflammation when tissue destruction is in progress offered the main evidence of the autoimmune nature of the disease. The major role of cell-mediated cytotoxicity in tissue destruction was shown and the finding of autoantibodies further indicates an autoimmune nature of the disease.

To study the response to long-term enzyme replacement treatment, a survey comparing the general well-being of 76 German shepherd dogs and rough-coated Collies treated for EPI and 145 healthy control dogs of the same breeds was performed (Study IV). The response to treatment with nonenteric-coated enzyme supplements, powdered enzymes or raw chopped pancreas, was found to be good with half of the dogs and they did as well as the healthy dogs. Despite the basically similar treatment regimens, the responses to treatment varied considerably. A poor response was found in a fifth of the dogs showing several signs of EPI. Although dietary sensitivities were common, the need for dietary treatment was minimal. Short relapses of clinical signs may develop during long-term treatment and, besides enzyme treatment, antibiotics were occasionally administered to half of the dogs during treatment. However, the study indicates that permanent deterioration of the clinical condition in dogs with EPI during long-term treatment is uncommon. Thus, the prognosis for long-term enzyme replacement treatment is considered generally to be good.

2. ABBREVATIONS

CFU	colony forming units
BSA	bovine serum albumin
BT-PABA	N-benzoyl-L-tyrosyl-para-aminobenzoic acid
GSD	German shepherd dog
ELISA	enzyme-linked immunosorbent assay
cEI	canine pancreatic elastase 1
EPI	exocrine pancreatic insufficiency
Ig	immunoglobulin
IM	intramuscular
IV	intravenous
kD	kilodalton
mAb	monoclonal antiboby
MCT	medium chain triglyceride
PAA	pancreatic acinar atrophy
PBS	phosphate buffered saline
p.o.	peroral
RED	radial enzyme diffusion
RER	rough endoplasmic reticulum
RIA	radioimmunoassay
RCC	rough-coated Collie
SEPI	subclinical EPI
SDS-PAGE	sodium-dodecyl-sulfate polyacrylamide gel electrophoresis
SIBO	small intestinal bacterial overgrowth
SST	soybean stimulation test
cTLI	canine trypsin-like immunoreactivity
TST	TLI stimulation test

3. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which are referred in the text by their Roman numerals (I-IV).

I Wiberg M.E., Nurmi A-K., Westermarck E. (1999a) Serum trypsin-like immunoreactivity measurement for the diagnosis of subclinical exocrine pancreatic insufficiency in dogs. *Journal of Veterinary Internal Medicine 13, 426-432*

II Wiberg M.E., Saari S.A.M., Westermarck E. (1999b). Exocrine pancreatic atrophy in German shepherds and rough-coated Collies: An end-result of lymphocytic pancreatitis. *Veterinary Pathology 36, 530-541*

III Wiberg M.E., Saari S.A.M., Westermarck E., Meri, S. (2000) Cellular and humoral immune responses in atrophic lymphocytic pancreatitis in German shepherd dogs and rough-coated Collies. *Veterinary Immunology and Immunopathology 76, 103-115*

IV Wiberg M.E., Lautala H-M, Westermarck E. (1998). Response to long-term enzyme replacement treatment in dogs with exocrine pancreatic insufficiency. *Journal of American Veterinary Medicine Association 1, 86-90*

4. INTRODUCTION

Chronic diseases of the exocrine pancreas may affect pancreatic secretory capacity and lead to inadequate production of pancreatic digestive enzymes and maldigestion signs typical for exocrine pancreatic insufficiency (EPI). EPI is a functional diagnosis based on measuring decreased pancreatic secretion capacity by a pancreatic function test (Freudiger 1971, Lankisch 1993, Williams 2000).

In dogs, the possible underlying pathological processes that may result in clinical signs of EPI are pancreatic acinar atrophy (PAA), chronic pancreatitis and pancreatic neoplasia (Thordal-Christensen and Coffin 1956, Geyer et al 1968, Holroyd 1968, Freudiger 1971, Rimaila-Pärnänen and Westermarck 1982, Williams 2000). The prevalences of these different pancreatic diseases in dogs are difficult to assess because pancreatic morphological examination is needed for the specific diagnosis. However, pancreatic acinar atrophy is reported to be by far the most common cause of severe EPI in dogs. Chronic pancreatitis is less commonly diagnosed in dogs, although it has been reported to be a common cause of EPI in humans and in cats. Compared to PAA, the clinical signs of chronic pancreatitis in dogs are more non-specific gastrointestinal signs and are sometimes associated with signs of endocrine pancreatic dysfunction. In conjuction with pancreatic neoplasia, EPI is a rarely reported clinical sign (Thordal-Christensen and Coffin 1956, Geyer et al 1968, Holroyd 1968, Freudiger 1971, Rimaila-Pärnänen and Westermarck 1982, Hänichen and Minkus 1990, Steer et al 1995, Steiner and Williams 1997, Williams 2000).

Canine PAA is a unique disease compared to PAA in other animals or humans (Eppig and Leiter 1977, Leiter and Cunliffe-Beamer 1977, Sidhu and Tandon 1995, Durie 1997). PAA has been studied extensively for the past 50 years, ever since it was reported by Thordal-Christensen and Coffin (1956), Geyer et al (1968) and Holroyd (1968), and further described by Säteri (1975). Earlier, the terms juvenile atrophy, pancreatic collapse and pancreatic degenerative atrophy were also used to describe atrophic changes of the pancreas (Thordal-Christensen and Coffin 1956, Holroyd 1968, Hill et al 1971, Rimaila-Pärnänen and Westermarck 1982).

Characteristic for PAA is a selective destruction of the digestive enzyme producing acinar cells, which gives rise to severe maldigestion. An atrophied pancreas is thin and transparent

and the inflammatory reaction of atrophied parenchyma is usually absent or slight (Thordal-Christensen and Coffin 1956, Geyer et al 1968, Hill et al 1971, Säteri 1975, Hashimoto et al 1979, Rimaila-Pärnänen and Westermarck 1982). Although clinical signs and pathological findings of PAA are well-documented in literature, the etiopathogenesis of the atrophy process has remained obscure. PAA is most commonly found in young German shepherd dogs and rough-coated Collies. Of all the dogs diagnosed with clinical EPI, approximately 70% were German Shepherd dogs and 20% rough-coated Collies (Freudiger 1971, Westermarck et al 1989, Hall et al 1991). The prevalence of PAA within these two breeds is estimated to be approximately 1% (Freudiger 1976, Westermarck et al 1989).

German shepherd dogs and rough-coated Collies with PAA show similar genetic, clinical and pathological findings indicating a similar etiopathogenesis of the disease with these two breeds. In both breeds, PAA has shown to be inherited (Weber and Freudiger 1977, Westermarck 1980, Westermarck et al 1989, Moeller et al 2002). The problem with earlier etiopathogenetic studies is that they have been based on findings at the clinical phase of the disease. The exocrine pancreas has a large reserve secretory capacity and the clinical signs of maldigestion do not occur until 90% of secretory capacity is lost (DiMagno et al 1973). Severely atrophied acinar tissue has offered little in terms of etiopathogenesis.

An ability to diagnose exocrine pancreatic dysfunction before the clinical phase would provide an opportunity for further study of the pathogenesis of the disease. Over the years, several studies have focused on developing a sensitive diagnostic test for exocrine pancreatic dysfunction in dogs. The value of these tests has rested on their ability to distinguish whether the signs of maldigestion are due to pancreatic disease or disease of the small intestine (Williams 2000). Today, the laboratory diagnosis of severe pancreatic dysfunction is reliable and simple and various function tests can be used to confirm the clinical diagnosis of EPI (Hill and Kidder 1970, Westermarck and Sandholm 1980, Batt and Mann 1981, Williams and Batt 1988, Spillmann et al 2001a). Further studies need to be carried out to ascertain whether these tests can also be used to detect early pancreatic dysfunction.

The treatment of dogs with clinical disease and signs of EPI includes supplementing their food with pancreatic enzymes. Several studies have been made to discover the most suitable formular of pancreatic enzymes for dogs and to evaluate the role of dietary modifications in the treatment of EPI (Pidgeon 1982, Pidgeon and Strombeck 1982, Westermarck 1987,

Westermarck et al 1990, Simpson et al 1994, Westermarck et al 1995). When severe PAA is the cause for clinical EPI, lifelong enzyme replacement treatment is needed. To date, no comprehensive studies on response to and prognosis for long-term treatment are available.

5. REVIEW OF LITERATURE

5.1 Pancreatic acinar atrophy (PAA)

5.1.1 Etiopathogenesis

In pancreatic acinar atrophy (PAA), there is selective destruction of the digestive enzyme producing acinar cells. Loss of acinar tissue leads to inadequate secretion of pancreatic enzymes and thus to maldigestion signs typical for EPI. The endocrine pancreas is usually unaffected. Although PAA is a well-recognized disease, the etiopathogenesis of the atrophy process has remained obscure (Thordal-Christensen and Coffin 1956, Geyer et al 1968, Holroyd 1968, Freudiger 1971, Hill et al 1971, Säteri 1975, Hashimoto et al 1979, Rimaila-Pärnänen and Westermarck 1982).

PAA has been reported in many breeds, but increasingly in German shepherd dogs and, in Finland, also in rough-coated Collies (Clark 1960, Geyer et al 1968, Freudiger 1971, Hill et al 1971, Säteri 1975, Prentice et al 1980, Rimaila-Pärnänen and Westermarck 1982, Williams and Batt 1988, Westermarck et al 1989, Hänichen and Minkus 1990, Boari et al 1994). PAA has been shown to be inherited both in German shepherd dogs and in rough-coated Collies. Pedigree analyses have suggested an autosomal resessive inheritance model (Weber and Freudiger 1977, Westermarck 1980, Westermarck et al 1989, Moeller et al 2002). Maldigestion signs typical for EPI appear usually at the age of 1 to 5 years and females and males are usually equally affected (Räihä and Westermarck 1989).

Compared to other species, canine PAA is a unique disease. Naturally occurring pancreatic acinar atrophy has been reported in CBA/J mice (Eppig and Leiter 1977, Leiter and Cunliffe-Beamer 1977). In humans, pancreatic acinar atrophy has been reported, but in association with multi-organ diseases such as Sjögren's and Shwachman-Diamond syndromes (Sidhu and Tandon 1995, Durie 1997).

Experimental studies have shown that acinar atrophy can be an end-result of various processes affecting the exocrine pancreas. These include pancreatic duct obstruction, ischemia, toxic situations, nutritional deficiencies or imbalances and defective secretory and/or trophic stimuli

(Herman and Fitzgerald 1962, Nevalainen and Janigan 1974, Fell et al 1982, Mizunuma et al 1984, Oates et al 1986, Walker et al 1993, Tanaka et al 1994, Sidhu and Tandon 1995, Watanabe et al 1995). However, there is no evidence to support the involvement of these factors in naturally occurring PAA in dogs (Washabau 1995, Williams 2000). Congenital isolated deficiencies of pancreatic enzymes have been reported in humans (Durie 1997) but not in dogs.

Because of the young age of the affected dogs, PAA has been also suggested to be a hypoplastic disease (Holroyd 1968, Jubb 1983). There are some reports describing congenital exocrine, or compound exocrine and endocrine pancreatic hypoplasia in young puppies (Boari et al 1994, Neiger et al 1996, Boari et al 1997). However, based on the morphological findings at the end-stage phase of the disease, the general opinion has been that PAA is a degenerative disease (Thordal-Christensen and Coffin 1956, Freudiger 1971, Hill et al 1971, Säteri 1975, Hashimoto et al 1979, Pfister et al 1980, Rimaila-Pärnänen and Westermarck 1982, Hänichen and Minkus 1990, Hellman and Loppnow 1991). Westermarck et al (1993a) followed morphological changes in the pancreas of a German shepherd dog puppy bred from parents with PAA. The results supported the hypothesis that acinar atrophy in German shepherd dogs is not hypoplastic, but due to a progressive and degenerative disease. Ultrastructural findings were first detected at the age of 6 weeks, but gross and histological abnormalities were seen only at the age of 2 years, when the clinical signs of EPI appeared and severe acinar atrophy was evident. The ultrastructural changes were degenerative without inflammation (Westermarck et al 1993a).

Role of autoimmunity in the pathogenesis of PAA

The possibility that acinar atrophy is a result of immune-mediated tissue destruction has previously received only scant attention and, to date, studies on the possible autoimmune nature of PAA are limited (Säteri 1975, Rimaila-Pärnänen and Westermarck 1982, Hellmann and Loppnow 1991, Simpson and Cobb 1998).

Autoimmune diseases are multifactorial. Genetic suspectibility, environmental factors, and immunological abnormalities are all involved in this pathogenesis (Sinha et al 1990, Rose and Bona 1993, Janeway et al 1999). To prove that a disease is autoimmune-mediated, it must be shown that an adaptive immune response to a self-antigen causes the disease pathology. It is

often difficult to find direct proof of autoimmunity and, often, only circumstantial evidence for the autoimmune nature of a disease can be shown. This evidence can include the following genetic, environmental, morphological and immunological factors that are characteristic for autoimmunity (Rose and Bona 1993, Janeway et al 1999).

Genetic susceptibility to autoimmune disease involves many genes and the strongest associations have been seen with the major histocompatibility class II genotype (Sinha et al 1990, Roep and De Vries 1992, Janeway et al 1999). Autoimmune diseases typically develop at a relatively young age and, because of hormonal influence, there is a female predominance (Sinha et al 1990, Janeway et al 1999).

Environmental factors, either microbial or non-microbial, are usually needed to initiate a clinical autoimmune disease in genetically susceptible individuals (Janeway et al 1999). In many cases, because of difficulties in proving their presence and role, the triggering factors can only be suspected. Although an autoimmune disease usually seems to occur spontaneously, there is a strong association between infection and the onset of autoimmunity. This suggests that infectious agents, especially viruses, could have a critical role in the process (Schattner and Rager-Zisman 1990, Rose and Bona 1993, Janeway et al 1999).

Autoimmune diseases may be divided into those mediated primarily by either cellular or humoral mechanisms (Sinha et al 1990, Roep and De Vries 1992, Rose and Bona 1993, Janeway et al 1999). Cell-mediated autoimmune reaction is characterized by lymphocyte infiltration into the organ, where tissue destruction is in progress (Carnaud and Bach 1993, Rose and Bona 1993). The evaluation of cell-mediated immune responses includes immunophenotyping the lymphocytes infiltrating the tissue and assessing the roles of different pathways and inflammatory cells (Sinha et al 1990, Janeway et al 1999). The basic screening of the humoral immune status includes serum protein electrophoresis, quantification of serum levels of different immunoglobulin classes or subclasses and serum autoantibody analyses (Sinha et al 1990). Most autoimmune diseases have a characteristic pattern of autoantibody production (Sinha et al 1990; Janeway et al 1999). Only a few autoantibodies such as anti-glomerular basement antibodies in Goodpasture's syndrome are the direct cause of clinical manifestations (Roitt el al 1998). More commonly, the autoantibodies are used as markers for the disease and predictors of the clinical outcome (Rose 1989, Sinha et al 1990, Roep and De Vries 1992, Roep 1996, Janeway et al 1999).

In humans, an immune-mediated etiology has been proposed for some exocrine pancreatic diseases. Pancreatitis and pancreatic insufficiency of an autoimmune nature have been reported in association with multi-organ autoimmune diseases such as Sjögren's syndrome, primary biliary cirrhosis and primary sclerosing cholangitis (Chari and Singer 1994, Ito et al 1997). Antipancreatic antibodies have been detected in some forms of pancreatitis, thus suggesting the involvement of immunological mechanisms in their pathogenesis (Lendrum and Walker 1975, Lankisch et al 1981, Rumenssen et al 1985). Recently, the existence of autoimmune-mediated chronic pancreatitis in humans has been suggested (Yoshida et al 1995, Ito et al 1997, Okazaki et al 2000). Findings indicative of an autoimmune nature included hypergammaglobulinemia, antipancreatic autoantibodies (antilactoferrin against acinar cells and anticarbonic anhydrase II against ductal cells), pancreatic lymphocyte infiltration with fibrosis and treatment response to steroids (Yoshida et al 1995, Ito et al 1997, Okazaki et al 2000).

In dogs with PAA, earlier studies have shown genetic and morphological evidence suggestive of an immune-mediated disease (Freudiger 1971, Säteri 1975, Weber and Freudiger 1977, Westermarck 1980, Rimaila-Pärnänen and Westermarck 1982, Westermarck et al 1989). However, a problem has been the lack of morphological and immunological studies from the period when tissue destruction is in progress. Also, the possible contribution of different environmental factors, such as feeding, housing, training, stress or viruses in the pathogenesis of PAA has been proposed, but there are no comprehensive studies on their roles. A survey failed to show any common triggering environmental factors in the histories of dogs with EPI (Räihä and Westermarck 1989).

Morphological studies in the clinical phase of PAA have shown the presence of lymphocytes and plasma cells in the atrophied pancreas, but their role has remained unclear because the samples were from the end-stage phase of the disease where the inflammatory reaction is usually mild (Hill et al 1971, Säteri 1975, Hashimoto et al 1977, Rimaila-Pärnänen and Westermarck 1982). Occasionally, larger amounts of lymphocytes have been detected in those clinical cases of PAA where tissue destruction was still partly in progress (Säteri 1975). This indicates that the cellular immunity may have become activated in the earlier phase of the atrophy process. Until now, only one study on the possible role of humoral immunity in the pathogenesis of PAA has been published. Simpson and Cobb (1998) reported circulating

pancreatic antibodies in dogs with EPI. However, since similar antibodies were found in healthy control dogs, their significance remained unclear.

5.1.2 Clinical signs of exocrine pancreatic insufficiency (EPI)

Clinical signs of the end-stage PAA are typical severe maldigestion signs of EPI (Freudiger 1971, Räihä and Westermarck 1989). In the study by Räihä and Westermarck (1989), the most typical clinical signs of EPI included yellowish or grey faeces, increased faecal volume and defecation frequency, weight loss and flatulence. These signs were present in about 90% of dogs. Abnormally increased appetite, poorly digested, loose and pulpy faeces and occasional coprophagia were found in every other dog. Nervousness or aggressiveness was also reported in one third of the dogs. These were suspected as resulting from abdominal discomfort due to increased bowel movements and gas formation (Räihä and Westermarck 1989). Severe watery diarrhoea is usually only temporary (Räihä and Westermarck 1989, Williams 2000). Prolonged and non-specific gastrointestinal signs may sometimes precede the typical clinical signs of EPI (Williams 2000). However, usually pre-existing gastrointestinal signs are not detected and signs of EPI develop within a short time frame in previously healthy animals (Räihä and Westermarck 1989). Skin disorders; poor coat and exemas have also been reported in dogs with EPI (Freudiger 1971).

Although the clinical signs of EPI are considered typical, they are not pathognomonic for exocrine pancreatic dysfunction. The differential diagnosis includes diseases of the small intestine, causing signs of malabsorption or maldigestion (Säteri 1975, Rimaila-Pärnänen and Westermarck 1982, Räihä and Westermarck 1989, Williams 2000).

5.1.3 Pathology

PAA shows typical gross pathological findings, which may already be considered pathognomonic for the disease. Grossly, the pancreas is normal in length but thin and transparent. No fibrotic or hemorrhagic tissue is found. The normal glandular structure is hardly recognizable and the pancreatic ducts are clearly visible (Thordal-Christensen and Coffin 1956, Geyer et al 1968, Hill et al 1971, Säteri 1975, Hashimoto et al 1979, Rimaila-Pärnänen and Westermarck 1982). Histologically, in the end-stage phase no normal acinar tissue is left, or if normal tissue is present, it is found in small isolated lobuli. Typically, the

normal acinar parenchyma is replaced by atypical tissue; disorganized small round cells with a central nucleus and light acidophilic, often slightly granular cytoplasm, and with prominent and dilated ductal structures. Fibrous tissue is not generally increased and in some cases the normal tissue has become replaced by adipose tissue. Inflammatory cells, lymphocytes and plasma cells may be found scattered or in small infiltrations among atrophied tissue (Thordal-Christensen and Coffin 1956, Geyer et al 1968, Hill et al 1971, Säteri 1975, Hashimoto et al 1979, Rimaila-Pärnänen and Westermarck 1982). The endocrine part of the pancreas in dogs with PAA is usually well preserved (Hill et al 1971, Säteri 1975), although some evidence for degenerative changes also in the endocrine cells has been reported (Pfister et al 1980, Hellmann and Loppnow 1991).

Säteri (1975) divided histological findings in the clinical phase of PAA into three stages. In the first stage, some sparse normal acinar tissue was found among the atrophic parenchyma and, in the border zone between the normal and the atrophied tissue, the gradual transition of acinar cells was associated with lymphocytic infiltration. In the second stage, atypical atrophic tissue dominated but some normal acini were still found. The inflammatory reaction was, however, less intense than during the earlier stage. In the end-stage atrophy ductular structures were prominent but no normal tissue or inflammation was present. Hellmann and Loppnow (1991) divided PAA cases into those with focal atrophy, atrophy associated with lymphocytic inflammation, and advanced atrophy of end-stage phase.

Only a few studies of the ultrastructural changes of PAA have been published. Hashimoto et al (1979) and Pfister et al (1980) described degenerative changes in the remaining acinar cells in the dogs with end-stage PAA. The findings were characterized by whorl formation and dilatation of the rough endoplasmic reticulum (RER) and loss of the zymogen granules. Westermarck et al (1993a) was dividing the progressive degenerative changes of acinar cells into four stages. The first stage showed the slight dilatation of RER. In the second stage RER became more dilated and disorganized, mitochondria and zymogen granules were larger than normal, and round condensing vacuoles were found in the cytoplasm. The nucleus was crenated and the chromatin condensed. In third stage dilatation of RER was increased, the nucleus was pyknotic, and there were extensive fusion of zymogen granules. In the final stage, acinar cells were necrotic.

Pathological findings in end-stage PAA are clearly different from those of chronic pancreatitis in dogs. Unlike in PAA, both the exocrine and the endocrine pancreas may become affected in chronic pancreatitis. Macroscopically, the pancreas is usually hard, shrunken and nodular, and there may be adhesions. Characteristic histological findings in chronic pancreatitis involve an increase in the interlobular and intralobular fibrosis and disorganized acinar lobuli, with or without inflammatory cells in the interstitium. Chronic pancreatitis may be classified as chronic interstitial pancreatitis, chronic fibrotic pancreatitis, pancreatic atrophic cirrhosis and pancreatic fibrosis (Thordal-Christensen and Coffin 1956, Geyer et al 1968, Holroyd 1968, Freudiger 1971, Rimaila-Pärnänen and Westermarck 1982, Hänichen and Minkus 1990).

5.2 Evaluation of exocrine pancreatic function

Either direct or indirect function tests are employed in evaluating the functional capacity of the exocrine pancreas. Direct tests measure the contents of pancreatic secretions in the duodenum, whereas indirect tests measure either pancreatic enzymes in the blood and faeces, or diminished digestive capacity (Lankisch 1993). The direct secretin-pancreozymin/ cholecystokinin stimulation test is considered the golden standard test for diagnosing and staging pancreatic dysfunction in humans. This test requires intubation of the patient, enterohormonal stimulation of the pancreas, and aspiration of the pancreatic juice from the duodenum to analyze the volume, bicarbonate and enzyme content. The particular advantage of direct tests is in diagnosing mild and moderate dysfunctions, but they are expensive, invasive, time-consuming, and impractical to perform (Lankisch 1993). Moreover, in performing direct tests on dogs, a general anaesthesia is needed. This may affect the secretory capacity of the pancreas and thus limit its value as a diagnostic test in canine pancreatic disease (Tiscornia et al 1972, Säteri 1975, Boden et al 1977).

Indirect tests are a practical alternative for diagnosing EPI and their diagnostic value increases along with the severity of pancreatic dysfunction (Lankisch 1993). In dogs, the value of pancreatic function tests has relied mostly on their ability to distinguish whether the clinical maldigestion signs are due to exocrine pancreatic dysfunction or to a small intestinal disease (Williams 2000). Various indirect function tests; such as canine serum trypsin-like immunoreactivity (cTLI) measurement, faecal enzyme measurements (faecal proteolytic activity, faecal elastase), and a digestion test (serum BT-PABA; N-benzoyl-L-tyrosyl-para-aminobenzoic acid) have been shown to be valuable in confirming the diagnosis of clinical

EPI (Hill and Kidder 1970, Westermarck and Sandholm 1980, Batt and Mann 1981, Williams and Batt 1988, Spillmann et al 2001a).

5.2.1 Serum trypsin-like immunoreactivity (TLI)

Recent years have seen the measurement of serum canine trypsin-like immunoreactivity (cTLI) by radioimmunoassay becoming one of the most commonly used pancreatic function tests to diagnose canine EPI. Serum TLI measurement is species- and pancreas-specific. It measures only pancreatic trypsin and trypsinogen that has entered the bloodstream directly from the pancreas (Williams and Batt 1983, 1988). Approximately 0.01% - 0.1% of the total daily secretion of trypsinogen leaks from acinar cells into the circulation (Borgström 1981). The reference range for cTLI in healthy dogs has been reported to be >5.0 – 35.0 µg/L (Williams and Batt 1988).

Serum cTLI measurement has proved to be very valuable in diagnosing severe exocrine pancreatic dysfunction in dogs. Abnormally low serum cTLI concentrations (< 2.5 µg/L) are found in association with the typical clinical signs of maldigestion. They are considered diagnostic for clinical EPI with high sensitivity and specificity (Williams and Batt 1988). Because trypsinogen does not need to be absorbed from the intestinal lumen, intestinal disease does not affect cTLI measurement. Enzyme replacement treatment does not affect serum cTLI measurement as the species-specific antibodies used in the assay do not cross-react immunologically with enzymes in supplementations and also because the absorption of the orally given enzymes into the bloodstream is limited (Williams and Batt 1988).

It is recommended that serum samples for TLI measurement are taken after fasting for 8 to 12 hours because a postprandial increase of serum trypsinogen levels, even slight and transient, may occur (Adrian 1980, Florholmen 1984, Reidelberg et al 1984, Williams 2000). A renal dysfunction associated with pancreatic disease could cause a rise in serum TLI because trypsinogen is eliminated by glomerular filtration (Geokas et al 1982, Williams 2000). In chronic pancreatitis, higher TLI values may be detected despite severe pancreatic dysfunction. This is because serum TLI can increase as a result of acute inflammatory attacks in the remaining exocrine tissue or because of pancreatic duct obstruction (Simpson et al 1989a, Keller 1990, Archer et al 1997).

In humans with chronic pancreatitis, the value of serum TLI for diagnosing mild or moderate dysfunction is considered questionable because TLI values overlapping with normal concentrations have been detected (Andrian 1980, Gullo et al 1980, Ruddell et al 1981). On the other hand, there have also been reports suggesting that serum TLI may be useful for an early diagnosis of exocrine pancreatic dysfunction both in humans and dogs**.** Andriulli et al (1981) reported that in human chronic pancreatitis, serum TLI concentrations correlated with results from the direct secretin-cholecystokinin test showing a parallel decrease with bicarbonate and enzyme secretion. In dogs, low serum cTLI values have reported to be the first marker of pancreatic dysfunction and low value may precede the clinical phase of EPI in dogs with PAA (Westermarck et al 1993a, Boari et al 1994). Simpson et al (1992) showed in dogs with partial pancreatectomy, and Westermarck et al (1993a) with naturally occurring PAA, that at the time the dogs showed no signs of EPI, the serum cTLI values were low but the other pancreatic function tests were still normal. Correlation analyses between serum cTLI and pancreatic weight have shown that about 25% of the normal pancreas mass is needed to maintain normal levels of serum cTLI (Simpson et al 1992).

Occasionally, subnormal serum cTLI values, i.e 2.5 - 5.0 µg/L have been detected in dogs. When these dogs were retested the cTLI values were either suggestive of EPI or within normal limits **(**Williams and Batt 1988). Clinical studies of the significance of serum cTLI values between 2.5 and 5.0 µg/L are, however, lacking, and it is unclear whether these values are indicators of subnormal exocrine pancreatic function (Williams and Batt 1988).

5.2.2 Serum TLI stimulation test (TST)

To increase the value of serum TLI measurement for diagnosing and staging exocrine pancreatic dysfunction, TLI has been measured before and after pancreatic stimulation with either endogenous or exogenous pancreatic stimulants. In humans and in dogs, endogenous stimulation by food has not been found useful. Although an increase in serum TLI was observed, the response was slow and affected by dietary factors (Adrian 1980, Bonora et al 1980, Spillmann 1995). A more significant response was achieved by exogenous stimulation with enterohormones, secretin and/or cholecystokinin (Adrian 1980, Bonora et al 1980, Vezzadini et al 1980, Spillmann 1995). Secretin is considered the main stimulant for bicarbonate fluid secretion and cholecystokinin for enzyme secretion, although some potentiated effect of two hormones has been reported (Beglinger et al 1984, Jo et al 1991).

In humans, the effect of secretin in increasing the serum TLI level has been suggested as being the result of an engorgement of the pancreatic ducts, which causes an increase in the intraductal pressure and regurgitation of trypsin into the bloodstream from the pancreas (Bonora et al 1980, Vezzadini et al 1980). In healthy humans, stimulation with secretin increased the serum TLI level, but no response to cholecystokinin stimulation was found (Adrian 1980, Bonora et al 1980, Vezzadini et al 1980). Vezzadini et al (1980) suggested that in patients with pancreatic disease the level of TLI response to secretin can depend on the stage of pancreatic dysfunction and could thus be used to assess the level of dysfunction.

The value of the serum TLI stimulation test for diagnosing EPI in dogs was studied by Spillmann 1995. In this study cTLI was measured by ELISA. When the pancreas of healthy dogs was stimulated either by a combination of both secretin and ceruletide (cholecystokinin analogue) or by ceruletide alone, a significant response was detected. While a response to stimulation occurred in normal dogs, no response was detected in pancreaectomized dogs. The stimulation test could therefore be helpful in diagnosing cases with untypical pancreatic insufficiency, or in cases where fasting serum cTLI values are unequivocal (Spillmann 1995). Spillmann et al (2000) studied TLI stimulation also in cats. Ceruletide stimulation with normal cats showed an increase, but whether that is a diagnostic value for evaluating pancreatic function in cats remained open.

5.2.3 Serum BT-PABA test

The serum BT-PABA (N-benzoyl-L-tyrosyl-para-aminobenzoic acid) test measures indirectly the level of pancreatic enzyme activity in the small intestine. The test is based on the hydrolysis of a perorally given synthetic peptide, BT-PABA, by chymotrypsin in the duodenum. The free PABA can be measured either from plasma or urine after it has become absorbed from the intestine into the blood (Lankisch 1993).

In dogs, the BT-PABA test has been shown to be valuable in diagnosing EPI (Batt et al 1979, Stradley et al 1979, Batt and Mann 1981, Zimmer and Todd 1985). The test is, however, impractical and the results may be affected by several factors such as delayed gastric emptying, hepatic or renal failure or by a severe malabsorptive small intestinal disease (Batt and Mann 1981, Zimmer and Todd 1985, Lankisch 1993). In humans, false normal BT-PABA results may be found in cases of mild or moderate pancreatic insufficiency (Lankisch 1993).

In dogs with an experimentally induced partial EPI, the serum PABA levels indicated a moderate dysfunction as the results were higher than in severe EPI but lower than in controls (Stradley et al 1979). On the other hand, when the BT-PABA test was compared to the serum cTLI measurement in dogs, the serum cTLI was found to be an earlier marker for EPI (Williams and Batt 1986, Westermarck et al 1993a).

5.2.4 Faecal enzyme measurements

Faecal proteolytic activity

Low faecal proteolytic activity has been reported in dogs with severe EPI (Hill and Kidder 1970, Burrows et al 1979, Westermarck and Sandholm 1980, Simpson and Doxey 1988, Williams and Reed 1990, Williams 2000). However, the reliability of different faecal proteolytic activity tests varies (Williams 2000). The gelatin digestion test is a simple qualitative/semiquantative method for detecting faecal proteolytic activity. The digestion of gelatin coating on X-ray film strips incubated in a faecal homogenate is suggestive of adequate faecal proteolytic activity. To increase the value of the X-ray film tests, it is important to standardize the technique by using X-ray films of the same type (Westermarck 1982). More reliable, semiquantitative methods for measuring faecal proteolytic activity are the azocasein method (Hill and Kidder 1970) and radial enzyme diffusion (RED) into agar containing a casein substrate (Westermarck and Sandholm 1980).

Faecal proteolytic activity should be measured in repeated samples because of the considerable daily variations in the results and because healthy dogs may occasionally pass faeces with low proteolytic activity (Hill and Kidder 1970, Westermarck and Sandholm 1980). To prevent false negative results, faecal proteolytic activity has been measured after giving the dogs raw soybean in the food and by measuring activities in faecal samples from 3 days (soybean stimulation test, Westermarck and Sandholm 1980). Pancreatic stimulation was shown to increase the sensitivities of both the X-ray and RED tests. After adding raw soybean to the food, a significant increase in faecal proteolytic activity was detected in apparently healthy dogs with low faecal protease activity, but not in dogs with severe EPI (Westermarck and Sandholm 1980). The mechanisms whereby soybean affects pancreatic secretion are, however, unclear. A feedback mechanism has been suggested; protease inhibitors in raw soybean are forming complexes with trypsin, thus decreasing the amount of proteases in the

intestine, and forcing the pancreas to further secrete (Anderson et al 1979). However, the natures of the feedback mechanisms in dogs are unclear and there are also reports that no pancreatic hypersecretion occurs after adding raw soybean to the food (Patten et al 1971).

Faecal elastase

A new faecal test for exocrine pancreatic dysfunction is the measurement of faecal elastase using the ELISA method. Elastase has been shown to have good intestinal stability, i.e. it does not degrade during the intestinal passage (Spillmann et al 2001b). Canine faecal elastase (cE1) is a species- and pancreas-specific test. No cross-reactions with bovine or porcine elastase have been detected. Thus the discontinuation of enzyme replacement therapy is not needed (Spillmann et al 2001b). The value of faecal elastase in confirming the diagnosis of clinical EPI has been shown both in dogs and in humans (Dominguez-Munoz et al 1995, Glasbrenner et al 1996, Löser et al 1996, Stein et al 1996, Spillmann et al 2001a).

5.3 Treatment of EPI

5.3.1 Enzyme replacement therapy

Because the clinical signs of EPI are due to the inadequate production of digestive enzymes, the primary treatment is to supplement each meal with pancreatic enzyme preparations. Despite accurate enzyme supplementation, digestion capacity does not return to normal. Only a small portion of the orally-given enzymes are delivered functionally intact into the small intestine (DiMagno et al 1973, Pidgeon and Strombeck 1982, Westermarck 1987). Various pancreatic enzyme extracts are available and the best results in dogs have been achieved by using nonenteric-coated supplements. The highest enzyme activity in the duodenum has been achieved by supplemention with raw chopped pancreas or with powdered enzymes (Westermarck 1987). The value of enteric-coated supplements has been shown to be limited in dogs because of the delayed gastric emptying of the preparations (Marvola et al 1986, Westermarck 1987). Preincubation of enzymes in food before feeding and supplementation with bile salts or antacids have been tried in a bid to increase the efficiency of enzyme supplementation, but with no proven efficiency (Pidgeon and Strombeck 1982, Hall et al 1991, Williams 2000). Inhibition of gastric acid secretion by the H_2- antagonist, cimetidine, has shown some positive effects (Pidgeon and Strombeck 1982). Because of the expense of

H_2-antagonists, their use is probably indicated only when the treatment response to enzymes alone is poor (Hall et al 1991, Williams 2000). Increasing the amount of enzyme supplement during the initial period of treatment or when there is an unsatisfactory response to treatment has been recommended (Hall et al 1991, Williams 2000). Other studies, however, have questioned the value of increased dosages (Pidgeon and Strombeck 1982, Westermarck 1987).

5.3.2 Dietary modification

Dietary modification with feeding a low fat, highly digestable and low fibre diet is commonly recommended for the treatment of EPI. However, clinical feeding studies have yielded controversial results of the actual benefits of using special diets (Pidgeon 1982, Batt 1990, Westermarck et al 1990, Hall et al 1991, Simpson et al 1994, Westermarck et al 1995).

Because enzyme supplements alone are unable to restore normal fat absorption, a low fat diet has been considered necessary to achieve a favourable response to treatment. Perorally-given lipase is easily destroyed by the acid conditions in the stomach and up to 90% of lipase activity is lost before lipase reaches the duodenum (DiMagno et al 1973, Pidgeon and Strombeck 1982). Fat absorption may be affected also by the bacterial deconjugation of bile salts in a small intestinal disease, producing metabolites which in turn may result in diarrhoea (Batt 1990, Simpson et al 1994). Simpson et al (1994) reported that during initial enzyme treatment the low fat diet was important for a satisfactory response to treatment. Westermarck et al (1995) studied the effect of a low fat diet during the treatment by comparing the clinical signs when dogs were fed with their ordinary diet and a low fat diet. The results showed that feeding the low fat diet did not significantly alleviate clinical signs and was thus not found necessary.

Highly digestable diets are recommended because undigested carbohydrates may produce osmotic diarrhoea and act as substrates for intestinal bacteria (Batt 1990). Feeding with a high fibre diet was found to increase faecal fat excretion and increase flatulence in human patients with EPI (Dutta and Hlasko 1985). In vitro studies have shown that some types of fibre may impair pancreatic enzyme activity (Dutta and Hlasko 1985). Clinical feeding studies in dogs with EPI have demonstrated that highly digestable, low fibre diets can alleviate clinical signs such as flatulence, borborygmi, increased faecal volume and defecation frequency (Westermarck et al 1990). Highly digestable diets may be of particular value in the initial

treatment until the nutritional status has improved and possible mucosal damage has been repaired. They may also be useful for dogs that fail to regain normal body weight with ordinary diets (Batt 1990, Williams 2000).

To increase the energy uptake of dogs with EPI, medium chain triglycerides (MCT) have also been added to their food. A recent study found no obvious clinical benefits in the use of MCT (Rutz et al 2001). Dietary sensitivities may be a consequence of EPI. Therefore, hypoallergic diets may benefit some dogs with EPI, especially in the early stages of treatment (Batt 1990).

5.3.3 Supportive therapy

EPI may be associated with secondary problems that may worsen the clinical signs. These include small intestinal bacterial overgrowth, malabsorption of cobalamin and the coexistence of a small intestinal disease (Batt and Morgan 1982, Simpson et al 1989b, Williams et al 1987, Rutgers et al 1995, Williams 2000).

Small intestinal bacterial overgrowth (SIBO) is common in dogs with EPI not only before enzyme replacement treatment has been started, but also during treatment (Williams et al 1987, Westermarck et al 1993b). An increased amount of substrates for bacteria in the small intestinal lumen, a lack of bacteriostatic factors of the pancreatic juice and changes in intestinal motility and immune functions are possible reasons for an abnormal accumulation of bacteria in the small intestine in dogs with EPI (Williams et al 1987, Simpson et al 1990, Johnston 1999).

The diagnosis of SIBO is based on the bacterial culture of the duodenal juice. The criterium for SIBO has been proposed to be $> 10^5$ CFU (colony forming units)/mL bacteria in the duodenum, although there is some disagreement on the definition of cutoff values (Batt 1983, Williams et al 1987, Simpson et al 1990, Westermarck et al 1993b, Delles et al 1994, Williard et al 1994, Rutgers et al 1995, Johnston 1999). Techical problems with taking duodenal juice samples have led to use indirect tests such as the measurement of serum folate and cobalamin, breath hydrogen testing and the measurement of deconjugated bile acids to detect SIBO (Batt and Morgan 1982, Johnston 1999, Melgarejo et al 2000). Serum folate and cobalamin measurements are based on the fact that some bacteria may synthesize folate and cause elevated serum folate levels and other bacteria may bind cobalamin and thereby prevent its

absorption, which decreases the serum levels. Although the tests measuring serum folate and cobalamin are practical, they lack sensitivity and specificity. Thus the finding of high serum folate and low cobalamin levels should be used only as evidence to support the occurrence of SIBO (Batt and Morgan 1982, Rutgers et al 1995, Johnston 1999). Recently it has been suggested that instead of SIBO, the term antibiotic responsive diarrhoea should be used when the clinical signs respond to antibiotic therapy, especially if there is no evidence of highly increased numbers of small intestinal bacteria (Johnston 1999).

Antibiotics have been shown to be a valuable supportive treatment for EPI. They have been used especially during initial treatment in cases of poor treatment response to enzymes alone and if the clinical signs have recurred during enzyme treatment. Antibiotics reported as effective are tylosin, oxytetracyclin and metronidazole (Williams et al 1987, Hall et al 1991, Westermarck et al 1993b, Rutgers et al 1995, Williams 2000).

Cobalamin deficiency has been reported in 36% to 76% of dogs with EPI **(**Batt and Morgan 1982, Hall et al 1991, Williams 2000**).** This deficiency is partly due an increased uptake of cobalamin by intestinal bacteria and partly because of the lack of the pancreatic intrinsic factor, which has been shown to have a major role in the absorption of cobalamin. Enzyme supplementation alone has not been found to be helpful for increasing serum cobalamin levels. Parenteral cobalamin treatment is needed when low serum cobalamin values are detected. However, the clinical signs of cobalamin deficiency are still poorly documented in dogs suffering from EPI (Batt and Morgan 1982, Batt et al 1989, Simpson et al 1989b, Williams 2000).

When treatment responses to enzymes and supportive therapies are insufficient, co-existing small intestinal disease and lymphoplasmacytic enteritis have to be suspected. To date, no comprehensive studies on EPI and associated small intestinal diseases have been published and recommendations for treatment with glucocorticoids are usually based only on personal observations (Williams 2000).

5.3.4 Prognosis

When the clinical signs of EPI appear in dogs with PAA, the loss of functional pancreatic tissue is already almost total. The changes are considered to be irreversible, and lifelong enzyme replacement treatment is usually required (Williams 2000). The few studies on the prognosis of PAA and on the response to treatment with enzyme supplements have focused on the response to initial treatment (Hall et al 1991, Simpson et al 1994). The overall response to enzyme treatment has been reported to be good in 64% and poor in 17% of cases, when the effect of treatment was assessed according to its ability to decrease diarrhoea and polyphagia and increase bodyweight (Hall et al 1991). In many dogs, a resolution of these signs could be found during the first weeks of treatment (Hall et al 1991, Williams 2000).

A reduction in enzyme doses may be tried after an initial response to treatment has been achieved (Hall et al 1991, Williams 2000). Decreasing the enzyme dose to 50% did not result in a significant deterioration of the clinical signs (Westermarck et al 1995). Although lifelong treatment is indicated for most dogs with PAA, there are also exceptions. In two dogs with clinical signs of EPI, the discontinuation of enzyme supplements after a good initial treatment response did not cause a recurrence of clinical signs (Westermarck and Rimaila-Pärnänen 1989b). Although these dogs had apparent pathological changes typical for PAA, some pancreatic tissue with reserve secretory capacity had remained and continuous enzyme replacement treatment was unnecessary.

Based on the survey by Hall et al (1991), during the first two years after the diagnosis of EPI, approximately 30% dogs were euthanized, some of the dogs immediately after diagnosis was established. The principal reasons for euthanasia were a failure to respond to treatment, refusal by the owners to continue treatment and the high cost of treatment (Hall et al 1991). Westermarck and Rimaila-Pärnänen (1989a) reported about severe, but rare complication of EPI; mesenteric torsion. This is probably caused by disorders in intestinal motility and by excessive intestinal gas.

6. AIMS OF THE STUDY

The aims of the current study were

I to study the etiopathogenesis of PAA

a) setting up a diagnostic method to detect exocrine pancreatic dysfunction before end-stage PAA and before the clinical signs of EPI have developed,

b) analyzing morphological changes in the exocrine pancreas during the progression of PAA,

c) assessing the roles of the cellular and humoral immune mechanisms in the pathogenesis of PAA and

II to study the response to long-term enzyme replacement treatment in dogs with clinical signs of EPI

7. MATERIALS AND METHODS

7.1 Animals

Studies I-III were set up to study the etiopathogenesis of PAA and to find a method for early detection of EPI, in the subclinical phase. Subclinical EPI (SEPI) was suspected in the dogs with a serum cTLI concentration < 5.0 µg/L (normal range, > 5.0 – 35.0 µg/L, Williams and Batt 1988), but no typical clinical signs of EPI. During 1995-1997, 1,236 serum samples were measured for cTLI in the Veterinary Laboratory Vetlab, Tampere, Finland. Of these samples, 187 were abnormally low (< 2.5 µg/L) and 76 subnormal 2.5 - 5.0 µg/l. Screening the data from 158 dogs with serum cTLI $\leq$ 5.0 µg/L revealed 44 dogs with low serum cTLI concentration but no clinical signs of EPI (Study I). Nine of the 44 dogs had cTLI concentration < 2.5 µg/L but no signs of EPI. In 35 dogs with various clinical histories the cTLI values were subnormal. These 44 dogs were considered as suspected of SEPI and were subjected to further studies (Tables 1, 2, Pages 39,40). Based on the pancreatic morphological findings (Study I and II), the diagnosis of SEPI/partial PAA was made in 13 the dogs (Tables 1-2, Pages 39,40). Findings of the dogs with SEPI were compared to those of the control dogs and the dogs with clinical signs of EPI (Study I, II and III). The control dogs (n=18) were apparently healthy dogs with a single serum cTLI concentration within the normal range (> 5.0 – 35.0 µg/L). A morphological examination of the pancreas by autopsy was performed in five laboratory beagles revealing a normal pancreas. Dogs with clinical EPI (n=23) had clinical maldigestion signs typical for EPI (weight loss, polyphagia, loose and voluminous faeces). The dogs had a single abnormally low serum cTLI (< 2.5 µg/L) value or low faecal proteolytic activity (Westermarck and Sandholm 1980) indicating severe pancreatic dysfunction. In 12 dogs, pathological examination verified end-stage PAA. The study was approved by the Ethical Committee of Faculty of Veterinary Medicine, University of Helsinki, and the owners allowed to use their pets in the study.

Study IV was set up to study the response to long-term enzyme replacement treatment in dogs with clinical EPI. The survey included 76 German shepherd dogs and rough-coated Collies with clinical EPI and 145 apparently healthy control dogs of the same breeds (Table 1, Page 39). EPI was diagnosed on the basis of clinical signs typical for EPI and on low serum cTLI concentrations (< 2.5 µg/L). Data on the control dogs was received from the breed associations. Based on the information received from the owners, the study included those control dogs that had no chronic illness necessitating continuous medication.

7.2 Methods

7.2.1 Pancreatic function tests

7.2.1.1 Serum cTLI (I, II, III, IV)

In all serum samples measured for canine trypsin-like immunoreactivity, cTLI, a radioimmunoassay (Double Antibody Canine TLI, Diagnostic Products Corporation, Los Angeles, CA) was used (Williams and Batt 1988). Serum samples were collected after an overnight fast and stored at -20^{o}C before analysis. In Study I, repeated cTLI measurements was performed to 44 dogs with suspected SEPI.

7.2.1.2 Serum TST (I)

TST was performed on 10 controls, 12 dogs with SEPI and 6 dogs with clinical EPI. Serum TST was modified from the test described by Spillmann (1995). After overnight fasting, serum samples for cTLI assay were collected from each dog. Immediately thereafter, the pancreas was stimulated by the intravenous administration of both secretin (1.0 IU/kg; Secretin, Ferring Pharmaceuticals, Malmö, Sweden) and cholecystokinin (1.0 IU/kg; Cholecystokinin, Ferring Pharmaceuticals, Malmö, Sweden) diluted 1:10 with NaCl and infused over 1-2 minutes. A serum sample for the second cTLI-assay was collected 20 minutes after stimulation.

7.2.1.3 Serum BT-PABA test (I)

The serum BT-PABA (n-benzyol-l-tyrosyl-para-aminobenzoic acid) test was performed on 4 dogs with SEPI. After overnight fasting, the dogs were given 16.5 mg/kg BT-PABA in 10 ml water/kg p.o., and one and two hours later serum samples were taken and analyzed for PABA (Batt and Mann 1981).

7.2.1.4 Faecal proteolytic activity (I)

Faecal proteolytic activity measurement after soybean stimulation (soybean stimulation test, STT) was performed on 10 dogs with SEPI. The dogs were fed raw soybean mixed with food for 4 days and faecal samples were collected on days 2, 3 and 4. Faecal proteolytic activity was measured by both radial enzyme diffusion (RED) in a calcium caseinate agar and by an X-ray film test (Westermarck and Sandholm 1980).

7.2.2 Pancreatic biopsy (I, II,)

A pancreatic biopsy was taken by laparotomy in 11 dogs with suspected SEPI and 3 dogs with clinical EPI. For laparotomy, the dogs were premedicated with medetomidine (40 µg/kg IM) and anaesthesia was induced with propofol (0.5-1.0 mg/kg IV) and maintained on 1% to 2% halothane. The pancreas was evaluated grossly by two observers. A pancreatic biopsy < 5 mm in diameter was taken from the caudal part of the pancreas by ligation with 3-0 absorbable monofilament suture material. All dogs were given prophylactic antibiotics (amoxicillin 20 mg/kg) and analgesics (buprenorphine 0.01 mg/kg) during surgery. Recovery from surgery was uncomplicated in all dogs. A biopsy was taken by autopsy from 5 control dogs, 2 of 13 dogs with suspected SEPI and 9 of 12 dogs with clinical EPI.

7.2.3 Histopathology (II)

Pancreatic biopsies of 5 control dogs, 13 dogs with SEPI and 10 dogs with clinical EPI were studied histopathologically. The biopsies (2-3 mm in diameter) were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Sections (4 mm) were stained with hematoxylin-eosin.

Two observers performed blind histological evaluation. The following findings were evaluated: histologically normal versus affected parenchyma, degree of inflammation, inflammatory cell pattern (infiltrative, diffuse, focal, multifocal), localization of inflammatory cells and inflammatory cell types, localization and degree of fibrous connective tissue.

7.2.4 Ultrastructure (II)

Pancreatic biopsies from 5 control dogs, 11 dogs with SEPI and 4 dogs with clinical EPI were studied by electron microscopy. Immediately after the pancreatic biopsy, parts of the biopsies were cut into pieces of 0.5 to 1 mm^3 and fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.3). Afterwards, the pieces were postfixed in osmium tetroxide and embedded in epoxy resin. For light microscopic studies, the semithin sections (1 mm) were cut and stained with 1% toluidine blue to select appropriate areas for ultrathin sections. The ultrathin sections were cut on the basis of light microscopic findings. The sections were placed on grids, stained with uranyl acetate and lead citrate and were examined under a Jeol JEM 100 S transmission electron microscope.

7.2.5 Cell-mediated immunity (II, III)

7.2.5.1 Immunohistochemical study

Formalin-fixed pancreatic biopsies were used for immunohistochemistry in order to study the presence of T-lymphocytes, insulin-producing Langerhans β-cells and macrophages. Formalin-fixed and paraffin-embedded pancreatic biopsies were cut into sections of 4 mm, and deparaffinized.

T-lymphocytes

To study the presence of T-lymphocytes, immunohistochemical studies were performed with biopsies obtained from 5 control dogs, 12 dogs with SEPI and 8 dogs with clinical EPI. Indirect immunoperoxidase staining was performed using a commercial streptavidin-biotin kit (DAKO LSAB Kit, Peroxidase; Dako Corporation, Carpinteria, CA). The sections were treated with 3% hydrogen peroxidase to inhibit the endogenous peroxidase. After blocking, the sections were incubated with a 1:50 polyclonal rabbit anti-human CD3 (cross-reacting with dog CD3) for 30 minutes (DAKO rabbit anti-human CD3, Code A454) to detect T-cells. Staining was done using the biotinylated secondary antibody and peroxidase-labelled streptavidin and completed after incubation with a substrate-chromogen solution (DAKO AEC Substrate System, Code K696). The sections were counterstained with Mayer's hematoxylin.

Double staining for T-lymphocytes and insulin producing β - cells

Double staining to detect T-lymphocytes and endocrine Langerhans β-cells was performed on sections from one control dog, 5 dogs with SEPI and one dog with clinical EPI. Staining was initially performed as described above with a 1:50 anti-human CD3 antibody, followed by incubation with a secondary antibody (DAKO Multilink, Code E453), peroxidase-conjugate (DAKO ABComplex/ HPR Code K355) and a substrate-chromogen solution (DAKO DAB Chromogen tablets, Code S3000). Insulin staining was done with a 1:200 anti-porcine insulin antibody for 30 minutes (DAKO guinea pig anti-porcine insulin, Code A464), a secondary antibody (DAKO Multilink), an alkaline phosphatase-conjugate (DAKO ABComplex/AP, Code K376) and a substrate-chromogen solution (DAKO Fast Red, Code K597). The sections were counterstained with Mayer's hematoxylin.

Macrophages

The presence of macrophages was studied in 4 control dogs, 8 dogs with SEPI and 4 dogs with clinical EPI. Indirect immunoperoxidase staining was performed by using a DAKO EnVision+, Peroxidase Kit (DAKO). After treatment with a peroxidase blocking agent, the sections were incubated with a 1:800 diluted rabbit anti-human lysozyme antibody for 30 minutes (DAKO rabbit anti-human lysozyme), followed by incubation with the peroxidase labelled polymer conjugated to secondary antibody. Staining was completed by incubation with a DAB substrate-chromogen solution. Counterstaining was done with Mayer's hematoxylin.

CD4+ helper T-cells, CD8+ cytotoxic T-cells, B-lymphocytes

Immunohistochemical analysis of the cryostat sections of pancreas was employed to detect CD4+ T-helper cells, CD8+ cytotoxic T-cells, B-lymphocytes and plasmacells. The pancreas biopsies (2-3 mm in diameter) from 5 control dogs, 9 dogs with SEPI and 4 dogs with clinical EPI were frozen in liquid nitrogen and stored at -80°C. Serial cryostat sections (4 µm) were prepared and stored at –20°C. Before use, the sections were kept at room temperature (22°C) for 15 minutes, fixed in cold acetone (+4 to +8°C) for 10 min and air dried at 22°C for 10 minutes.

Indirect immunoperoxidase staining was performed by using a commercial streptavidin-biotin kit (DAKO LSAB Kit, Peroxidase; DAKO). After blocking, the sections were incubated for 30 minutes at 22°C with one of the following primary antibodies: mouse anti-dog CD4 and CD8; diluted 1:20 (from Dr. Peter Moore, Leukocyte Antigen Biology Lab, University of California, Davis, CA) and mouse anti-human CD79; diluted 1:50 (DAKO). The bound antibodies were detected with the biotinylated secondary antibody, peroxidase-labelled streptavidin, and a substrate-chromogen solution (DAKO AEC Substrate System). The sections were counterstained with Mayer's hematoxylin.

Evaluation of immunohistological findings

Two observers performed blind evaluation. Estimates of the degree, pattern and localization of CD3-positive cells were obtained by comparing the CD3-stained slides with the hematoxylin-eosin-stained slides. The number and distribution of macrophages were estimated in non-affected, inflamed and atrophied tissues. The number of cells staining positively with CD4, CD8 and CD79 mAbs were counted using a light microscope at 400 x magnification. The average number of positive cells in 15 to 30 representative fields of 0.79 x 0.79 mm were counted for each staining. The intensity of lymphoid cell infiltration was estimated by semiquantitative analysis as follows: 0= no positively staining cells, 1+=< 5 positive cells; 2+= 5 to 20 positive cells; 3+ = 20 to 50 positive cells; 4+ = > 50 positive cells/field. The inflammatory cell pattern was described as: scattered= infiltrating single cells; multifocal= numerous separate cellular infiltrates; focal= larger lymphoid cell aggregates with or without resemblance of a lymphoid follicle; diffuse = extensive cell infiltration.

7.2.6 Humoral immunity (III)

7.2.6.1 Serum protein electrophoresis

The serum samples from 10 control dogs, 8 dogs with SEPI and 6 dogs with clinical EPI were analyzed by standard agarose gel electrophoresis (Beckman Paragon, Beckman Instruments, Fullerton, CA).

7.2.6.2 Serum immunoglobulin quantification

Serum IgG, IgM, IgA concentrations from 5 control dogs, 6 dogs with SEPI and 8 dogs with clinical EPI were determined using a single radial immunodiffusion method (SRID Kit, Canine IgG, IgM, IgA, Veterinary Medical Research and Development, Pullman, WA) and standards provided by the manufacturer.

7.2.6.3 Serum autoantibody detection

Immunofluorescence microscopy

Indirect immunofluorescence microscopy was used to search for any pancreas-specific and non-organ specific antibodies in the sera from 13 control dogs, 9 dogs with SEPI and 10 dogs with clinical EPI. Cryostat sections (4 μm) were prepared from tissue samples from a non-diseased dog's pancreas, liver, kidney, gastric mucosa, oesophagus, adrenal gland and submandibular salivary gland. Before use, the sections were put in room temperature (22°C) for 15 minutes, fixed in acetone (+4 to +8°C) for 10 min and air dried at 22°C for 10 minutes.

The sections were treated with 3% BSA in phosphate-buffered saline (PBS) for 5 minutes to prevent non-specific binding reactions. The sera were diluted 1:2 with 3% BSA/PBS to detect pancreatic antibodies and 1:5 to detect non-organ specific antibodies. The sections were incubated with serum samples for 30 minutes at 22°C. After washing, the fluorescein-conjugated secondary antibody (goat anti-dog IgG, Southern Biotechnology Associates, Birmingham, AL), diluted 1:20 was added and incubated for 30 minutes. After washing, the sections were mounted with the Mowiol mounting medium and analyzed under an Olympus BX50-FLA microscope using a filter specific for fluorescein.

Antinuclear antibodies were analyzed using human epithelioid cells (HEp-2 cells, American Type Culture Collection, Bethesda, MD). The cell plates were incubated with sera diluted 1:20 with PBS for 30 min at 22°C. After washing, the fluorescein-conjugated secondary antibody (goat anti-dog IgG) diluted 1:20 was added and incubated for 30 min at room temperature. After washing, the samples were analyzed as above.

Immunoblotting analysis

The Western blotting method was used to analyze potential pancreas-specific antibodies in sera from 7 control dogs, 7 dogs with SEPI and 7 dogs with clinical EPI. A pancreatic tissue sample from a non-diseased dog was homogenized, heated to 56°C for 5 minutes and solubilized in SDS buffer in the presence of a non-reducing buffer or reducing buffer containing β-mercaptoethanol (10%). Proteins in the pancreatic homogenates were separated by SDS-PAGE in 12.5% gels (Mini-PROTEAN II Dual Slab Cell, Bio Rad Laboratories, Richmond, CA) using molecular weight markers from Biorad (SDS-PAGE Standards, Low Range). For immunodetection, the separated proteins were transferred electrophoretically from the gel into a nitrocellulose membrane. The nitrocellulose strips were incubated at 22°C overnight with the blocking solution (1% BSA in Tris-saline-Tween [Tween-20, 0.05 %]) and, thereafter, with the sample sera diluted 1:100 with 1% BSA-Tris-saline-Tween for 1 hour at 22°C. After washing, the strips were incubated for 1 hour at 22°C with alkaline phosphatase-conjugated secondary antibody (Rabbit anti-dog IgG, Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:500 with Tris-saline-Tween. After further washing, staining was completed using a chromogen substrate for alkaline phosphatase (AP Color Development Reagent BCIP and NBT, Bio Rad). Samples were analyzed after a short rinse in water.

7.2.7 Survey of the response to long-term enzyme replacement treatment (IV)

Questionnaires were sent to the 145 owners of dogs with clinical EPI (German shepherd dogs or rough-coated Collies) and to 211 owners of clinically normal control dogs (German shepherd dogs or rough-coated Collies). Dogs with EPI had been given dietary enzyme supplements for at least 4 months. Detailed questions about current clinical signs and dietary habits were asked. Owners were asked to assess severity and generality of gastrointestinal and dermatological signs, using criteria provided with the questionnaire. Owners of the dogs with EPI were also asked about enzyme supplements, dietary modification and adjunctive treatments during the initial and long-term treatment periods.

7.2.8 Statistical methods

Study I: In TST**,** a t-test for paired samples was used to study the significance of the TLI response in each group. Differences between the groups were tested with Student's t-test for unequal variances. A p-value of < 0.05 was considered significant.

Study III: One-way analysis of variance followed by Tukey's test was used to study the differences in serum electrophoretic fractions and serum immunoglobulin levels between the three groups: control dogs, dogs with SEPI and dogs with clinical EPI. A p-value of < 0.05 was considered significant.

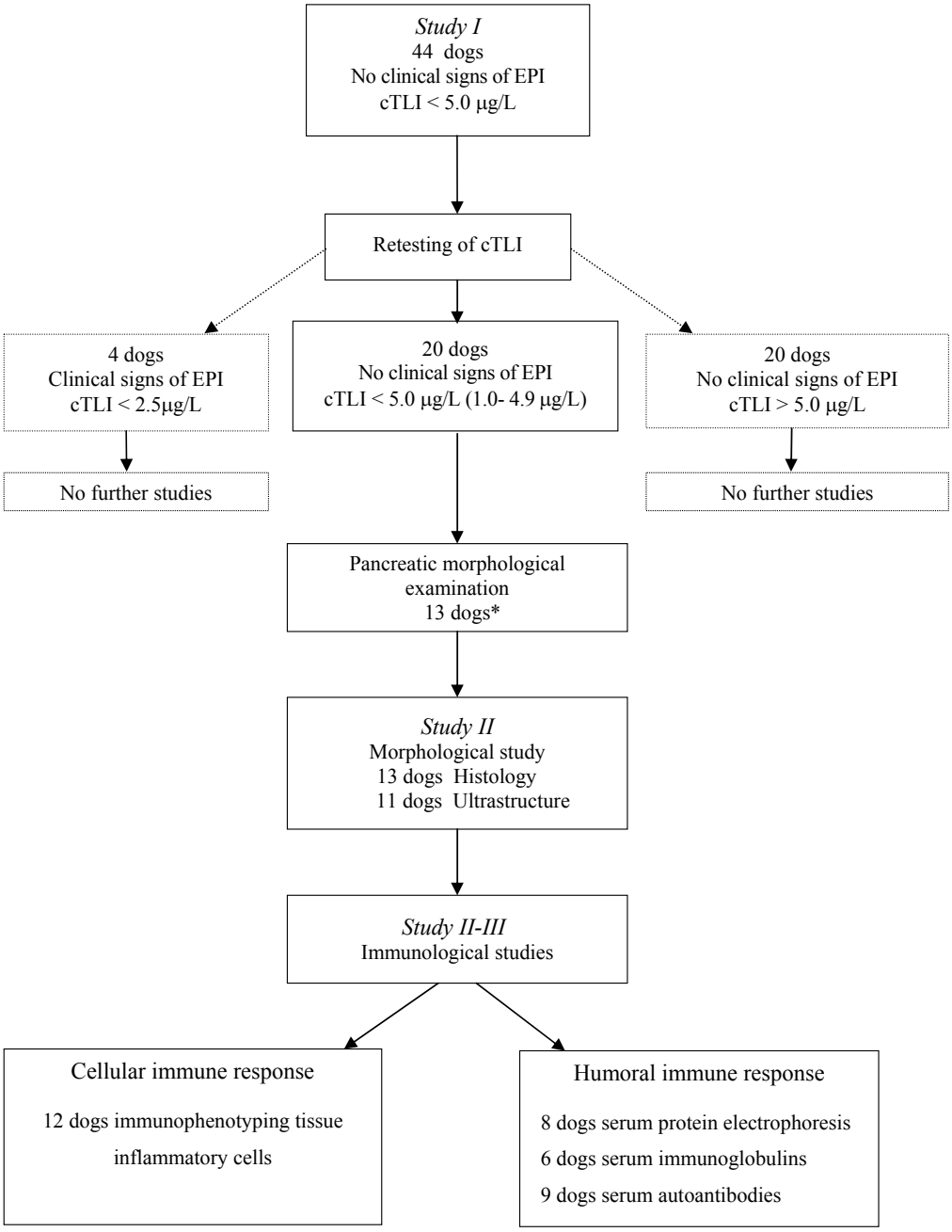
Study IV: Multinomial logit models and cross tabulations followed by χ^2 testing were used to compare the relative frequency distributions of gastrointestinal tract and dermatological signs, feeding regimens and dietary intolerances between EPI- and control-group dogs. Eleven gastrointestinal signs (Table 3, Page 51) were classified as typical for dogs with EPI on the basis of clinical signs of the dogs in Study IV and information in the previous report (Räihä and Westermarck 1989). Prevalences of typical EPI signs were compared between the different groups by means of logit models and cross tabulations followed by χ^2 testing. The models were evaluated by the use of likelihood ratio goodness-of-fit statistic. Tukey's test was used to compare the number of typical EPI signs among the groups of dogs treated for various periods. A p-value of < 0.05 was considered significant.

Table 1. Study categories and characteristics of animals in the different study groups

Study	Purpose of the study	Study group	Breed, age, sex	Criteria
I	Diagnostic study for subclinical EPI/partial PAA	Suspected subclinical EPI n=44	27 GSD, 7 RCC, 10 other breeds Mean age 3.3 years 25 M, 19 F	No clinical signs of EPI Single cTLI < 5.0µg/L PAD (n=12)
		Clinical EPI n=6	5 GSD, 1 RCC Mean age 3.5 years 4 M, 2 F	Typical clinical signs of EPI Single cTLI < 2.5µg/L PAD (n=3)
		Control dogs n= 10	10 beagles, Mean age 1 years 6 M, 4 F	Apparently healthy Single cTLI > 5.0–35.0 µg/L PAD (n=5)
II	Morphological Immunological study	Subclinical EPI/ Partial PAA n=13	11 GSD, 2 RCC Mean age 3.2 years 8 M, 5 F	No clinical signs of EPI cTLI repeatedly < 5.0µg/L *
		Clinical EPI/PAA n=11	10 GSD, 1 RCC Mean age 6.3 years 7 M, 4 F	Typical clinical signs of EPI Single cTLI < 2.5µg/L (n=5) Low faecal prot. activity (n=6)
		Control dogs n=5	5 beagles Mean age 1 year 3 M, 2 F	Apparently healthy Single cTLI > 5–35.0µg/L
III	Immunological study	Subclinical EPI/ Partial PAA n=12	10 GSD, 2 RCC Mean age 3 years 7 M, 5 F	No clinical signs of EPI cTLI repeatedly < 5.0µg/L PAD (n=12)
		Clinical EPI n=13	11 GSD, 2 RCC Mean age 3.7 years 11 M, 2 F	Typical clinical signs of EPI Single cTLI < 2.5 µg/L (n=11) Low faecal prot. activity (n=2) PAD (n=4)
		Control dogs n=13	8 GSD, 5 beagles; Mean age 1.1 year (n=8) 9 M, 4 F	Apparently healthy Single cTLI > 5.0–35.0µg/L PAD (n=5)
IV	Treatment study	Clinical EPI n=76	45 GSD; age 5.3 years 31 RCC; age 4.6 years 45% M, 55 % F	Typical clinical signs of EPI cTLI < 2.5 µg/L
		Control dogs n=145	72 GSD; age 3.5 years 73 RCC, age 3.6 years 46 % M, 54% F	Apparently healthy

EPI= exocrine pancreatic insufficiency; PAA= pancreatic acinar atrophy; Breeds; GSD=German shepherd dog; RCC=rough-coated Collie;M=male, F=female; cTLI= fasting serum canine trypsin-like immunoreactivity measurement (RIA) Criteria: based on clinical status, serum cTLI or faecal proteolytic activity measurement, and PAD results (pathologic-anatomic diagnosis). *In one dog with partial PAA (Study II, III), a single low cTLI value was taken

Table 2. A flowchart showing the diagnostic methods of the Studies I-III.



cTLI= fasting serum canine trypsin-like immunoreactivity (radioimmunoassay)
* For one of the dogs, a single cTLI measurement (< 5.0 µg/L) was taken

8. RESULTS

8.1 Diagnosis of subclinical EPI caused by partial PAA (I)

8.1.1 Serum cTLI

To study the value of serum cTLI measurement in diagnosing exocrine pancreatic dysfunction in the subclinical phase, 44 dogs with suspected subclinical EPI were retested for cTLI. The dogs had a low serum cTLI < 5.0 µg/L but not typical signs of EPI.

In retesting, cTLI was repeatedly low (<5.0 µg/L) in 20/44 dogs (16 German shepherd dogs, 4 rough-coated Collies; ages 1 to 8 years, mean 3.7 years; 11 males and 9 females). Clinically, 15 dogs had no gastrointestinal signs at the time of retesting, and 5 dogs had signs not typical for EPI (e.g., occasional diarrhoea, food intolerance). Laparotomy and pancreatic biopsy were performed on 12/20 dogs (10 German shepherd dogs and 2 rough-coated Collies, aged 1 to 8 years; mean 3.4 years, with 7 males and 5 females). At the time of laparotomy, serum cTLI of these dogs ranged from 1.0 to 4.1 µg/L (mean, 2.7 µg/L). The time periods between the first cTLI measurement and laparotomy ranged from 1 to 12 months (mean, 4.6 months). In all 12 dogs the laparotomy verified partial acinar atrophy (detailed results are shown in 8.2.). Because the dogs with partial PAA did not show signs typical for EPI, they were diagnosed as having subclinical EPI (SEPI).

By the time of retesting, clinical EPI was confirmed in 4/44 dogs (3 German shepherd dogs and 1 Chow Chow; aged 1 to 5 years, mean 2.5 years; all females). In retesting, these dogs showed clinical signs of EPI and serum cTLI concentrations ranged from 0.6 to 0.9 µg/L (mean, 0.7 µg/L). The times between the TLI measurements ranged from 2 to 12 months (mean, 5.5 months). No further studies were performed on these dogs.

Retesting showed cTLI values in the normal range in 20/44 dogs (8 German shepherd dogs, 3 rough-coated Collies, 2 Great Danes, 2 Chow Chows, 5 representing different breeds; aged 1 to 9 years, mean 4.2 years; 14 males, 6 females). Serum cTLI concentrations ranged from 5.2 to 28.4 µg/L (mean, 10.5 µg/L). The time intervals between the measurements ranged from 1 to 27 months (mean, 18.4 months). Clinically, 13 dogs showed no gastrointestinal signs at the time of retesting, 6 dogs had occasional gastrointestinal signs and 1 dog had chronic diarrhoea. No laparotomy was performed on any of these dogs.

8.1.2 Serum BT-PABA and faecal proteolytic activity

An evaluation was made of the value of serum BT-PABA and faecal proteolytic measurement in diagnosing subclinical EPI. The results of serum BT-PABA testing were subnormal in 3 of 4 dogs with SEPI and normal in one dog. Mean peak PABA level was 20.5 µmol/L (range, 11.5 to 32.2 µmol/L; normal range, > 25 µmol/L). The results of faecal proteolytic activity measurement by SST showed normal faecal proteolytic activity (peak, > 6 mm calsium caseinate agar; range from 11.0 to 21.6 mm, mean, 17.4 mm) in all 10 dogs with SEPI when tested with radial enzyme diffusion (RED). The result using X-ray films was analogous with the RED assay (positive) in all except 1 of the 10 dogs.

8.1.3 Serum TST

Figure 3, Study I summarizes the results of the TLI-stimulation tests (TST). Control dogs (n=10) showed a significant increase in the mean (±SE) cTLI concentration from 10.3 ± 0.52 µg/L (range, 7.4 to 12.6 µg/L) to 35 ± 10.96 µg/L (range, 13.0 to 114.8 µg/L) after secretin and cholecystokinin stimulation. No significant increase in cTLI levels occurred in dogs with clinical EPI (n=6). In these dogs mean (±SE) fasting cTLI concentrations were 1.0 ± 0.24 µg/L (range, 0.3 to 1.9 µg/L) before and 1.2 ± 0.28 µg/L (range, 0.4 to 2.3 µg/L) respectively after stimulation. The 12 dogs with confirmed SEPI showed a significant response to TST. In this group the mean (±SE) fasting cTLI was 2.7 ± 0.28 µg/L (range, 1.0 to 4.1 µg/L) and after stimulation 22.2 ± 5.71 µg/L (range, 4.7 to 61.2 µg/L). The extent of response varied from dog to dog but in all except 1 dog, the cTLI concentrations increased to > 5.0 µg/L after TST. The response to stimulation was significantly higher in both control dogs and dogs with SEPI than in dogs with clinical EPI. No difference was observed in TST between dogs with SEPI and control dogs.

8.2 Morphological findings during progression of PAA (I, II)

Pancreatic gross pathological, histological and ultrastructural studies were carried out on dogs with SEPI and partial PAA. The results were compared to findings seen in dogs with clinical EPI due to severe PAA and to those in healthy control dogs.

8.2.1 Gross pathology (I)

In control dogs, normal pancreas was found. In dogs with SEPI, gross examination showed partial atrophy of the pancreas; the pancreatic mass was diminished and although the pancreas was normal in length it was thinner than a normal one. Within the normal tissue, there were scattered areas that had lost their glandular appearance. These changes were similar in all dogs but varied in extent. No signs of firm, nodular, or haemorrhagic tissue were observed (Fig.2, Study I). In dogs with clinical EPI, findings typical for severe acinar atrophy were found. The pancreas was thin, transparent and had lost its normal glandular tissue.

8.2.2 Histopathology (II)

Control dogs

In the control dogs, histologically normal exocrine and endocrine pancreas was found. Signs of inflammation were absent, except for a few individual lymphocytes within the ductal epithelium. Occasionally, in all five control dogs, isolated scattered apoptotic bodies were detected within the acinar cells. The judgment of apoptosis was based on the identification of membrane-bound apoptotic bodies, large enough to be recognized under a light microscope.

Subclinical phase

In 12 dogs of 13 with SEPI, the pancreatic sections revealed a histologically normal, partially affected and severely atrophied acinar structures. In one dog, the section contained only histologically normal acinar architecture. The main finding in dogs with SEPI was a marked lymphoid cell inflammatory reaction that was most extensive in the border zone area, where a gradual loss of normal acinar architecture was observed. These areas were defined as active border zones. The inflammatory cells had infiltrated from the active border zone into normal

acinar tissue. Numerous intra-acinar lymphocytes were detected in both histologically normal acini and partly affected acini (Fig. 1 and 2, Study II). Occasionally, several separate active border zone areas were found within the same lobules, giving the appearance of piecemeal tissue destruction. In addition, single lymphocytes or foci of lymphocytes were detected in otherwise histologically normal lobules. The predominating inflammatory cells were small and medium-sized lymphocytes. Besides lymphocytes, plasma cells and macrophages were also found. Plasma cells were seen more frequently in the areas of more advanced tissue destruction. In four dogs, eosinophils were detected in the border zone area. In two dogs, focal mononuclear cell infiltration resembling lymphoid follicles were detected. The inflammatory reaction in the border zone in dogs with SEPI was not associated with any increase in the amount of fibrous connective tissue.

From the border zone areas towards the atrophied parenchyma, the inflammatory reaction became milder. In the atrophied parenchyma, the degree of inflammatory reaction varied, usually consisting of multifocal lymphocytes scattered among atrophied tissue. More extensive focal infiltrates were sometimes found. Intraepithelial lymphocytes were frequently found in the ductal epithelium. In two dogs, the sections included both active and inactive border zones. In the inactive border zone, there was a sharp demarcation line between normal and atrophied tissue, and no gradual destruction of the acini was found (Fig. 3, Study II).

Gradual loss of normal acinar architecture was always associated with inflammation. Subsequent to the loss of acinar cells, the remaining parenchyma was composed of disorganized cells and inflammatory cells. As tissue destruction continued, the ductal structures became more prominent. In atrophied tissue, there was a slight increase in both intralobular and periductal fibrosis.

In nine dogs with SEPI, apoptotic bodies were found in the cytoplasms of individual acinar cells, usually in otherwise histologically intact acini (Fig. 2, Study II). In some dogs, an increased number of apoptotic bodies were detected near the active border zone area, but usually no defined pattern was found.

Clinical phase

In 6 of 10 dogs with clinical EPI, the exocrine pancreatic tissue had become totally atrophied. In 4 dogs, isolated areas with intact acinar structure were also found. These areas were separated from atrophied parenchyma by a sharp demarcation line such as an inactive border zone. The inflammatory reaction in the atrophied parenchyma varied and was usually less prominent than in subclinical phase. The atrophic tissue consisted of disorganized cells, ductal structures and adipose tissue. Typically, in advanced cases, the ducts were clearly visible and varied from small cuboidal epithelium lined ducts to large dilated ones with columnar epithelium. Fibrosis was usually slightly increased in the clinical phase compared to the subclinical phase. However, tissue replacement by fat was more prominent than fibrosis (Fig. 4, Study II).

8.2.3 Ultrastructure (II)

Control dogs

The sections of the control dogs were shown to have normal acinar structure and usually cytological details of normal appearance (Fig. 8, Study II). In some acinar cells mitochondria had lamellar densities and inclusions. Inflammatory cells were rare or not present. Occasionally, single apoptotic bodies were identified within acinar cells.

Subclinical phase

In dogs with SEPI, the exocrine parenchyma was typically affected by a moderate to severe mononuclear cell inflammatory reaction. Characteristically, numerous lymphocytes were found infiltrating within the acinar basement membrane. These intra-acinar lymphocytes were found both in acini that revealed no other ultrastructural changes and in partially affected acini (Fig. 9, Study II). A few scattered lymphocytes were found within the ductal epithelium. Occasionally, macrophages were also seen within the acini and in the ductal epithelium.

In one dog with SEPI, the section contained no remaining acinar cells and the findings were similar to those of dogs in the clinical phase. In general, the pancreas sections of dogs with SEPI included both unaffected acini and acini showing various stages of degeneration. The acinar architecture was still identifiable in most of the sections. Slightly affected acinar cells had a mildly dilated RER and normal or mildly swollen mitochondria. The nuclei were unaffected and the zymogen granules were similar to those in normal acinar cells. Moderately affected cells showed increased RER dilatation and disorganization, and the homogeneous swelling of mitochondria. Numerous zymogen granules were still present. In the severely affected, necrotic cells, the RER was markedly dilated and vesiculated, the nuclei were pyknotic and cell sizes had decreased. The zymogen granules were less abundant, but normal in size and shape, and no fusion of granules was found (Fig. 10, Study II). Prozymogen granules, larger and less electron dense than zymogen granules, were found in some acini. In these acini, the lumen was usually dilated and filled with secretory material.

In addition to the gradual degenerative changes described, sometimes markedly changed mitochondria were observed in acinar cells, which showed no visible alterations in RER. These changed mitochondria showed lamellar densities and inclusions, similar to those of the control dogs. Occasionally, in otherwise normal or mildly affected acini, large autophagic vacuoles were found containing cell debris and unidentified material.

In some dogs with SEPI, apoptotic bodies were identified within the acinar cells. Typically, more than one apoptotic body was found, either in normal or affected acini. In some acini, both apoptotic bodies and intra-acinar lymphocytes were found (Fig. 9, Study II).

As the surrounding acinar cells were being gradually destroyed, the centroacinar cells and other ductal cells were becoming more prominent. Morphologically the centroacinar cells and ductal cells lining the intercalated ducts were comparable to those of the control dogs. The flat ductal cells had typically large, electron lucent granules in the cytoplasm (Fig.11, Study II). Occasionally in the partially destroyed parenchyma, duct-like structures were found, which comprised altered acinar cells and cells resembling ductular cells lining a dilated empty lumen. Acinar cells had decreased in height and had markedly dilated RER and swollen mitochondria. Zymogen granules of normal size and electron density were present, although decreased in number. The ductular cells mostly resembled centroacinar cells and flat ductular cells (Fig. 12, Study II).

In more the advanced stages of destruction, the acinar cells were rarely or if ever seen and the parenchyma consisted of ducts varying extensively in size and shape. The lumens of these ducts were usually markedly dilated. Small ducts were lined by a flat cuboidal epithelium and when the sizes of the ducts increased the epithelium gradually changed from cuboidal to columnar. There were numerous small electron dense granules in the apex of the columnar epithelial cells. Mitotic activity was not found in any of the ductal cells. The pancreatic islet cells became more prominent when the acinar parenchyma had been destroyed.

Clinical phase

In the pancreas sections from dogs with clinical EPI and in one dog with SEPI, no remaining acinar cells were detected. The sections showed an altered parenchyma in which ducts varying greatly in size, islet cells, fibroblasts, fibrocytes and increased adipose tissue were all identified. The inflammatory reaction was usually less severe than found in dogs with SEPI. Besides lymphocytes and macrophages, an increased number of plasma cells were found in the altered parenchyma. The islet cells were typically scattered among the ductal structures. Occasionally, the islet cells were found within the ductal epithelium, but usually not in a direct contact with the ductal lumen.

8.3. The role of cellular immune response in the pathogenesis of PAA (II, III)

The role of cellular immunity was studied by immunophenotyping the inflammatory cells infiltrating the tissue in control dogs, dogs with SEPI/partial PAA and dogs with clinical EPI/ end-stage PAA.

Control dogs

In the control dogs, a few or no lymphocytes expressing the CD3, CD4 or CD8 antigen were detected and no cells staining positively for the CD79 antigen were found in the pancreas sections. Positive staining for the lysozyme antigen was seen in a few granulocytes. Macrophages were not present.

Subclinical phase

In dogs with SEPI, the majority of the lymphocytes infiltrating the pancreas were CD3+ T-cells. Characteristically, the lymphocytes infiltrating between normal acini, intra-acini and within the ductal epithelium were CD3-positive (Fig. 5, Study II).

Further immunophenotyping of the lymphocytes showed that generally, CD4+ and CD8+ T-cells were present in almost equal numbers, but the distributions of the lymphocyte subsets were different. The CD4+ T-cells usually formed large cellular infiltrates and were multifocally distributed in the affected parenchyma. The CD8+ T-cells were more widely scattered throughout the parenchyma than the CD4+ cells. In sections where both normal and affected acinar parenchyma were present, a predominance of infiltrating CD8+ T-cells was found. In particular, lymphocytes infiltrating an otherwise histologically normal parenchyma were mainly CD8+ (Fig. 1, Study III).

Also numerous CD79+ cells representing B-lymphocytes and plasma cells were found in sections with marked T-cell infiltration. Plasma cells showed diffuse cytoplasmic staining and the rest of the CD79+ cells, considered as B-lymphocytes, showed membrane associated staining. The CD79+ cells had a tendency to form large cellular infiltrates, but were also found scattered within a partially affected parenchyma. In some sections, clusters of mononuclear cells resembling lymphoid follicle germinal centres were observed. These lymphoid follicles typically showed intense staining with the CD79 antibody. In some follicles, also CD4+ cells were found, but CD8+ cells were usually absent (Fig. 2, Study III).

Scattered lysozyme positive macrophages were present in the areas of lymphocytic infiltration and advanced destruction of acinar parenchyma. In the normal acinar, no parenchyma infiltrating macrophages were detected.

The Langerhans islet β-cells, which stained positively for insulin, were located in large groups among exocrine parenchyma in dogs with SEPI (Fig. 6, Study II). In the more severely atrophied parenchyma, the insulin-positive cells formed small irregular groups scattered among the ductal structures (Fig. 7, Study II).

Clinical phase

In dogs with clinical EPI and in the atrophied tissue of dogs with SEPI, the lymphocytic inflammatory reaction was usually mild to moderate. Most of the scattered lymphocytes were CD3- positive, and both CD4+ and CD8+ T-cells were detected. Only a few or no CD79+ B-lymphocytes or plasma cells were found. Some macrophages were present in the atrophied parenchyma.

8.4 The role of humoral immune response in the pathogenesis of PAA (III)

8.4.1 Serum protein electrophoresis and immunoglobulin levels

Serum protein electrophoresis revealed no monoclonal components in any of the serum samples tested. A tendency towards an increase in the γ-globulin fraction was detected in dogs with SEPI (6.8 ± 0.7 g/L; mean, ±SE) and clinical EPI (7.0 ± 0.7 g/L) when compared to the control dogs (5.1 ± 0.3 g/L; $p = 0.0557$). The α-1 globulin fraction was significantly ($p = 0.0075$) decreased in dogs with clinical EPI (5.4 ± 0.6 g/L) when compared to the control dogs (7.6 ± 0.2 g/L) and dogs with SEPI (8.0 ± 0.8 g/L).

Serum immunoglobulins were quantified by radial immunodiffusion. No significant differences were found in IgG, IgM or IgA concentrations when comparing control dogs, dogs with SEPI and dogs with clinical EPI ($p > 0.05$).

8.4.2 Serum autoantibodies

The indirect immunofluorescence method was employed to detect any serum autoantibodies against pancreatic tissue components. A weak positive staining reaction against a granular component of the acinar cell cytoplasm was found with a serum dilution 1:2 in 5/9 dogs with SEPI and in 3/10 dogs with clinical EPI, but not in the control dogs (Fig. 3, Study III). No reactions against the ductal epithelial cells were detected.

In an attempt to try identify a possible autoantigen, immunoblotting analysis was performed using dog sera and both non-reduced and reduced pancreatic homogenates as target antigen mixtures. No apparent differences between sera from the control dogs, dogs with SEPI or clinical EPI in the protein band patterns that stained positively were observed with either non-

reduced and reduced pancreatic homogenates. With both types of homogenates, interpretation of the results was affected by a blurring of the protein bands and strong background staining especially in the > 45-55 kD areas.

Positive results in serum antinuclear antibody testing were found in 3/13 of the control dogs, 3/9 of dogs with SEPI and 1/10 dogs with clinical EPI (at serum titres 1:20). A speckled immunostaining pattern was most commonly detected. Indirect immunofluorescence staining was used for the analysis of antibodies against liver, kidney, gastric mucosa, oesophagus, adrenal glands and salivary glands, but no differences was found between control dogs, dogs with SEPI and dogs with clinical EPI.

8.5 Response to long-term enzyme replacement treatment (IV)

Questionnaires were returned by 110 (76%) owners of dogs with EPI and 156 (74%) owners of control dogs. Of dogs with EPI, 80 were alive and 30 were dead at the time of the study. For statistical analysis, data were used from 145 healthy control dogs (72 German shepherd dogs and 73 rough-coated Collies), and 76 dogs with EPI (45 German shepherd dogs and 31 rough-coated Collies)(Table 1, Page 39.). The study included information of EPI-group dogs which were alive and fed (at the time of the survey) a nonenteric-coated enzyme supplement (powdered supplement, Viokase V, Fort Dodge Laboratories, Iowa, or raw chopped pancreas). Four dogs with EPI which were fed a different enzyme supplement were excluded from the study. The enzyme supplementation had lasted from 4 months to 1 year in 20% of EPI-group dogs, 1 to 2 years in 22%, 2 to 4 years in 45%, and more than 4 years in 13% of EPI-group dogs. Powdered enzymes were given to 40 dogs and raw chopped pancreas to 36 dogs. The use of enzyme supplements was similar between the two breeds.

A comparison of the relative frequency distributions of gastrointestinal signs between EPI- and control-group dogs revealed significant differences in all signs ($p < 0.05$) (Table 1, Study IV). The prevalences of the 11 gastrointestinal signs, classified as typical signs of EPI, were more common in EPI-group dogs, compared with control-group dogs (Table 3, Page 51.). No significant difference was found between dogs receiving powdered enzymes or raw chopped pancreas, nor when EPI-group dogs were compared on the basis of how long treatment had lasted. The difference between breed was significant for only in a few signs, such as coprophagia (Table 2, Study IV).

Table 3. Prevalences of typical signs in dogs with EPI during enzyme replacement treatment, compared with clinically normal dogs.

Clinical signs	% of dogs with EPI n = 76	% of control dogs n = 145
Yellow faeces	51	3
Increased faecal volume	40	12
Pulpy faeces	33	2
Coprophagia, sometimes	30	12
Indoor defecation, sometimes	25	11
Flatulence, often	29	1
Defecation frequency, > 3 times/day	23	4
Thinness	21	4
Ravenous appetite	21	4
Diarrhoea, ≥ 1 to 2 times/week	17	2
Borborygmus, often	12	1

Significant ($p < 0.05$) difference in all signs between EPI- and control dogs

The general response to treatment was estimated according to the number of typical signs of EPI in each dog. An excellent or good response was found in 47% of the EPI-group dogs. These dogs had no more than 2 of 11 typical clinical signs of EPI and they did as well as most (97%) of the control-group dogs. In 33% of dogs, a satisfactory response was found with 3 to 5 typical clinical signs. Poor response to treatment was found in 20% of EPI-group dogs that had 6 or more typical signs of EPI (Table 3, Study IV). The owners were asked to assess their dog's response to treatment by comparing the dog's current health with that before the first signs of EPI. Of the owners, 50% estimated that their dog's current health was similar, and 33% thought that at the time of the study their dogs were healthier.

In dogs receiving raw chopped pancreas, the pig pancreas was most commonly used (72%), followed by cattle (19%), and lamb or reindeer (9%). The amount of the raw pancreas offered was 87 ± 44 g/meal (mean, ±SD). The amount of powdered enzyme (Viokase V) given per meal was 3.0 ± 1.5 g. The initial amount of enzyme supplementation was increased in 11% of dogs with powdered enzyme and in 12% of dogs with raw pancreas. In 18% of the dogs on long-term treatment, the amount of powdered enzyme was decreased. The mean (±) estimated monthly costs were $38 ± $21 for powdered enzyme and $11 ± $8 for raw pancreas.

Problems were reported by two thirds of the owners feeding raw pancreas (eg, impracticability in everyday use and difficulties in availability) and by half of the owners feeding powdered enzyme (eg, cost, supply difficulties). During long-term treatment, the type of enzyme supplementation was changed in 32% of the dogs for various reasons (eg, the high price of the previous supplements, practical difficulties, persistent clinical signs).

When the enzyme treatment was started, dietary modifications were made in 80% of the dogs with EPI**.** In general, dietary modifications were made by changing the previous regular dry dog food to some other type of dry dog food (45%), or by starting to feed the dogs a homemade diet (28%). A special commercial diet was fed to 22% of the dogs. At the time of the study, EPI- and control-group dogs were fed various brands of regular dog food and various homemade diets. In general, the most commonly fed dry foods were similar in both groups. Homemade diets consisted mainly of rice, potato, meat, chicken and cottage cheese. Two dogs with EPI were fed a special diet continuously. Most EPI- and control group dogs had some variation in their daily dietary routines. Any sudden dietary changes resulted in unwanted clinical signs significantly more often in the EPI-group dogs (82%) than in the control group dogs (45%). Adverse reactions to various diets and dietary ingredients were seen significantly more often in the EPI-group dogs (77%), than in the control dogs (55%). Clinical signs, as reported by the owners, due to dietary intolerance were diarrhoea, flatulence, borborygmus and dermatological signs.

Besides enzyme supplements, other medications to treat gastrointestinal tract signs were used in 62% of EPI-group dogs. Of adjuctive treatments, antibiotics were used in 42% of the dogs during initial treatment and in 45% during long-term treatment. Tylosin was the most commonly used antibiotic. The most common indications for antibiotics were diarrhoea, flatulence and borborygmus. During long-term treatment the antibiotics were not usually used for more than a week and the course was repeated once or twice a year. Two dogs received tylosin daily. Of the other adjunctive medications, H_2-antagonist, cimetidine, was used in three dogs and in one of them continuously. Cobalamin, was administered parenterally to 41% of the dogs, usually once or twice during treatment, to improve general health and the coat.

Dermatological signs were found increasingly in the EPI-group dogs (46%) compared to the control-group (24%). The most common problem was excessive hair loss and exemia. The dermatological problems were more commonly found in the German shepherd dogs.

Besides data from 76 dogs with EPI alive at the time of study, data was analyzed from 30 dogs with EPI that were deceased before the study was initiated. Of these dogs, 2 had died naturally, 20 were euthanized during the first year after diagnosis (one of the dogs immediately after diagnosis was confirmed), and 8 were euthanized later. The most common reason for euthanasia was the persistence of clinical signs, which was the only reason for euthanasia in 13 dogs and one of the reasons in 7 dogs. The most common persistent signs were diarrhoea and weight loss.

9. DISCUSSION

9.1 Diagnosis of partial PAA

PAA is characterized by the progressive destruction of pancreatic acinar tissue, leading to inadequate secretion of pancreatic digestive enzymes and to the clinical maldigestion signs of EPI. Although PAA is a well-recognized disease, previous studies have failed to show the etiopathogenesis of the disease (Thordal-Christensen and Coffin 1956, Geyer et al 1968, Holroyd 1968, Freudiger 1971, Hill et al 1971, Säteri 1975, Hashimoto et al 1979, Rimaila-Pärnänen and Westermarck 1982). Diagnosis of exocrine pancreatic dysfunction in dogs has been largely based on the typical clinical signs of EPI. Nevertheless, when clinical signs of EPI appear in dogs with PAA, the atrophy of acinar tissue is already almost total, offering little in terms of pathogenesis. To study further the etiopathogenesis of PAA, the diagnosis should be set in an earlier phase, when the tissue destruction is still in progress.

Study I assessed whether the specific tests used for the evaluation of pancreatic function in dogs are valuable for diagnosing exocrine pancreatic dysfunction already in the subclinical phase, and whether these tests could be used as a diagnostic aid for the early detection of PAA.

In human medicine, exocrine pancreatic dysfunction can be diagnosed and staged to mild, moderate and severe forms by using a direct pancreatic function tests, such as secretin-cholecystokinin test. In this test the pancreatic juice is aspirated directly from the duodenum to analyze the volume, and bicarbonate and enzyme content (Lankisch 1993). In dogs, the direct tests are not a practical alternative for screening pancreatic dysfunction, as they are laborious, and have to be carried out in anaestesia, which may have a negative effect on pancreatic secretion (Tiscornia et al 1972, Säteri 1975, Boden et al 1977). Thus in dogs, indirect pancreatic function tests, such as serum cTLI, serum BT-PABA, faecal proteolytic activity and faecal elastase measurements are used for diagnosing EPI (Hill and Kidder 1970, Westermarck and Sandholm 1980, Batt and Mann 1981, Williams and Batt 1988, Spillmann et al 2001a).

Serum cTLI measurement has been widely used as a screening test for canine exocrine pancreatic dysfunction, as it has been shown to be both a sensitive and practical method for detecting severe EPI. Abnormally low serum cTLI values (< 2.5 µg/L, normal range > 5.0 – 35.0 µg/L) have been shown to indicate severe loss of pancreatic function and are found in dogs with clinical signs of EPI due to end-stage PAA (Williams and Batt 1988, Westermarck et al 1993a).

There are reports indicating that the cTLI measurement may be used in the early diagnosis of exocrine pancreatic dysfunction. It has been reported that occasionally serum cTLI values can be found in subnormal range of 2.5 - 5.0 µg/L (Williams and Batt 1988). Subnormal cTLI values may be considered suggestive for impaired pancreatic function. However, to date, no comprehensive studies have been published on the clinical significance of these values. It has also been shown that serum TLI concentration reflect both the secretory capacity and functional mass of the exocrine pancreas (Andriulli et al 1981, Simpson et al 1992), and that low serum values may precede the appearance of clinical signs of EPI in dogs with PAA (Westermarck et al 1993a, Boari et al 1994).

In Study I, the screening of the clinical histories of dogs with a single low serum cTLI (< 5.0 µg/L) revealed a number of dogs in which subnormal pancreatic function could be suspected. Dogs with a subnormal range cTLI (2.5 - 5.0 µg/L) were found rather frequently (6% of all serum samples measured for cTLI). Clinically, these dogs varied from apparently healthy dogs to those with various gastrointestinal signs. Subnormal pancreatic function was also suspected in those exceptional dogs with an abnormal low serum cTLI (< 2.5 µg/L) indicating severe pancreatic dysfunction but without any clinical evidence of EPI.

To study whether a low cTLI level in these dogs was an occasional finding or an indicator of pancreatic dysfunction, repeated cTLI measurements were performed (Study I). Interestingly, in retesting, in half of the dogs cTLI was repeatedly < 5.0 µg/L but the dogs still showed no signs of EPI, thus suggesting subnormal pancreatic function in these dogs. The suspected pancreatic disease was verified by laparotomy and pancreatic biopsy, showing partial PAA in all 12 dogs studied. The dogs were all either German shepherd dogs or rough-coated Collies. Both breeds are known to be predisposed to PAA (Weber and Freudiger 1977, Westermarck 1980, Westermarck et al 1989). When the pancreas still has enough reserve secretory capacity, dogs with partial PAA showed no clinical signs of exocrine pancreatic dysfunction.

Based on the results, it was concluded that repeatedly low serum cTLI values (< 5.0 µg/L) in a clinically healthy dog are a valuable marker of subclinical EPI and highly suggestive for partial PAA in German shepherd dogs and rough-coated Collies.

However, Study I showed also that using serum cTLI measurement to diagnose subclinical EPI has some limitations, since in some dogs a low serum cTLI was only an occasional finding. These dogs also included breeds other than German shepherd dogs and rough-coated Collies. In the absence of further examinations, i.e. laparotomy, it remained uncertain whether these dogs had a pancreatic disease or not. Therefore, repeated measurements are needed to increase diagnosis accuracy, especially when a single subnormal cTLI (2.5 - 5.0 µg/L) value is detected. It has been observed in human patients that overlapping TLI results can occur in patients with mild to moderate exocrine pancreatic dysfunction and those with normal pancreas (Andrian 1980, Gullo et al 1980, Ruddell et al 1981). In a progressive disease such as PAA in dogs, cTLI concentrations can be expected to vary from subnormal to normal depending of the degree pancreatic tissue loss. Further, a serum cTLI value within a normal range does not necessary exclude the possibility of milder forms of acinar atrophy, especially in breeds predisposed to PAA.

The diagnostic value of other indirect pancreatic function tests: BT-PABA test and faecal proteolytic activity after soybean stimulation (Westermarck and Sandholm 1980, Batt and Mann 1981,) to diagnose subclinical EPI was assessed in Study I. The results showed that serum BT-PABA and SST were insufficiently sensitive to detect SEPI, although they are both good indicators of clinical EPI (Westermarck and Sandholm 1980, Batt and Mann 1981, Williams and Batt 1986, Westermarck et al 1993a). The BT-PABA test may support the diagnosis of SEPI but is not specific for pancreatic disease and abnormal results have been found also in small intestinal disease (Batt and Mann 1981, Williams and Batt 1986). The BT-PABA test was performed on only a few dogs. Compared to the other tests, the BT-PABA test is both impractical and expensive. SST is a semiquantative test and its value for staging the level of dysfunction was limited. This was expected because dogs with SEPI have remaining pancreatic reserve secretory capacity and pancreatic stimulation with soybean will lead to results within the normal range. A comparison of the results of the BT-PABA test and SST with those of cTLI measurements showed that serum cTLI is more valuable in the early diagnosis of exocrine pancreatic dysfunction. Williams and Batt (1986) reported similar findings.

A new indirect pancreatic function test, canine faecal pancreatic elastase, has been shown to be valuable to confirm the diagnosis of severe clinical EPI (Spillmann et al 2001a). A recent study of the diagnostic value of the faecal elastase test to diagnose subclinical disease showed that although significantly lower faecal elastase values were detected in dogs with SEPI than in the control dogs, the overall practical value of the test for diagnosing and screening for subclinical EPI was found to be questionable (Wiberg et al 2001). This is because of the overlapping results in the SEPI and control dogs and because of remarkable day-to-day variations in faecal elastase. It may thus be concluded that, at the moment, serum cTLI measurement appears to be the most promising indirect test for the early diagnosis of exocrine pancreatic dysfunction.

In this study, the suspected pancreatic disease was verified by pancreatic morphological examination during laparotomy (Study I and II). Previous studies have shown that pancreatic biopsies can be taken without further complications by laparotomy dissecting a small pancreatic sample, or by laparoscopy using crushing forceps (Westermarck et al 1993a, Twedt 1999, Spillmann et al 2000, Harmoinen et al 2002). Also, in this study no complications were detected after taken the biopsies. Laparotomies in dogs with subclinical disease revealed in all dogs already grossly recognizable pancreatic changes, which varied with severity. Gross findings were similar, but not as severe as in end-stage PAA, showing scattered areas of atrophied tissue among normal glandular tissue. In dogs with partial PAA, the remaining secretory capacity of the pancreas depends on the degree of atrophy. However, because of the uneven distribution of atrophic changes, it was difficult to estimate the remaining normal pancreatic mass. Therefore, no correlation analyses between morphological changes and serum cTLI concentrations or the other pancreatic function tests were attempted (Study I).

To further assess the reserve secretory capacity of the remaining exocrine pancreatic tissue, it has been thought that the exogenous stimulation of the pancreas with enterohormones secretin and cholecystokinin may lead to different TLI stimulation reactions depending on the degree of pancreatic dysfunction (Spillmann 1995). In Study I, both secretin and cholecystokinin were used for maximal pancreatic stimulation. Secretin stimulates the secretion of pancreatic bicarbonate-rich fluid, which increases pressure in the pancreatic ducts and allows enzymes to leak into the bloodstream. On the other hand, cholecystokinin acts as a direct stimulant of enzyme secretion. Previously, TLI has been measured in humans, dogs and cats after various stimulation methods (Adrian 1980, Bonora et al 1980, Vezzadini et 1980, Spillmann 1995;

Spillmann et al 2000). Study I demonstrated that dogs with end-stage PAA and clinical EPI showed no response to enterohormonal stimulation in contrast to dogs with partial PAA and SEPI. This indicates that in the subclinical phase there is still some reserve secretory capacity in the pancreas despite the fact that some dogs with SEPI had low fasting values typical for clinical disease. However, the cTLI stimulation test was not found to be more valuable in diagnosing SEPI than repeated cTLI measurements. This is because the degrees of stimulation responses in dogs with SEPI and in the normal control dogs were similar, despite different fasting cTLI concentrations. Although in the subclinical phase, low fasting serum cTLI concentrations were usually increased to normal level after stimulation, this response was not seen in all dogs and the level of responses varied from dog to dog. The value of the TST in staging the level of dysfunction remained uncertain, as it was not possible to correlate the results of this test with the amount of remaining pancreatic tissue.

Study I showed that, based on the clinical findings and serum cTLI measurement, it is possible to diagnose EPI in the subclinical and clinical phase and in breeds predisposed to PAA, these findings are suggestive for partial PAA and end-stage PAA, respectively. Repeatedly low serum cTLI (< 5.0 µg/L) measurements in dogs showing no clinical signs of EPI were found to be a sensitive method for the detection of subclinical disease. However, cTLI is probably not useful for diagnosing mild dysfunction or for further staging the level of EPI. These limitations should be taken into consideration if cTLI measurement is planned to be used as a screening test for subclinical cases, e.g in breeding programmes. The future will show the value of the diagnostic criterium given by this study. It will also been seen whether similar findings are detected in breeds other than German shepherd dogs and rough-coated Collies.

9.2. Morphological findings during the progression of PAA

The ability to diagnose PAA before the development of total acinar atrophy in the subclinical phase offered new possibilities to study the etiopathogenesis of the disease. Previously, it has been able to follow the progression of PAA only in a single dog born from the test-mating of parents with PAA (Westermarck et al 1993a). Based on the findings of Study I, it was now for the first time possible to study the histological and ultrastructural findings in a larger number of dogs with partial PAA whilst tissue destruction was still in progress.

The main histological feature in the subclinical phase of PAA was a marked infiltration of lymphocytes into the exocrine pancreas in distinct contrast to the usually mild inflammatory reaction in the clinical phase (Study II). The inflammatory reaction affected only the exocrine pancreas and, in the absence of fibrosis, showed a distinct difference in the morphological changes in canine chronic pancreatitis (Thordal-Christensen and Coffin 1956, Geyer et al 1968, Rimaila-Pärnänen and Westermarck 1982, Hänichen and Minkus 1990). Lymphocytic inflammation was most extensive in the border zone areas of normal and partially affected tissue and lymphocytes had spread into the normal acinar parenchyma. Typical findings included numerous intra-acinar lymphocytes, piecemeal appearance of tissue destruction and the presence of large groups of lymphocytes resembling lymphoid follicle germinal centres. The gradual destruction of acinar tissue was associated with inflammation. In areas of more advanced tissue destruction, the findings were similar to those found in end-stage PAA, where acinar structure is replaced by atypical parenchyma, ductular structures, and adipose tissue. Based on the findings in Study II, the progression of acinar atrophy was divided into lymphocytic pancreatitis with active destruction of the acinar structures and end-stage PAA. Thus, the term "atrophic lymphocytic pancreatitis" is preferred to describe the pathological findings.

Morphological findings during the progression of acinar atrophy indicated the autoimmune nature of the disease. Lymphocyte infiltration into the target organ where tissue destruction is in progress has been taken as primary evidence for autoimmune reaction (Carnaud and Bach 1993, Rose and Bona 1993). Findings in the subclinical phase of PAA were similar as reported for autoimmune diseases such as Hashimoto's thyroiditis in humans and lymphocytic thyroiditis both in humans and in dogs (Beierwaltes and Nishiyama 1968, Fritz et al 1970, Gosselin et al 1981, Gosselin et al 1982, Lucke et al 1983, Conaway et al 1985, Beale 1991). Characteristics for autoimmune diseases is also that the susceptibility to disease is multifactorial, including both genetic and environmental factors (Rose and Bona 1993, Janeway et al 1999). PAA has been reported to be inherited in German shepherd dogs and rough-coated Collies (Weber and Freudiger 1977, Westermarck 1980, Westermarck et al 1989, Moeller et al 2002), thus supporting the hypothesis of an autoimmune disease. All the dogs diagnosed with partial PAA were either German shepherd dogs or rough-coated Collies. Some of these dogs were known to have full siblings with the clinical disease (Study I and II). To date, the studies have failed to identify any common environmental factor predisposing to PAA (Räihä and Westermarck 1989). The ability to diagnose the disease before severe

atrophy may now offer further possibilities to study and identify the possible factors involved in triggering the progression of atrophy.

Previously, only scant attention was given to the possibility of PAA being an autoimmune disease. One reason for this was that although some inflammatory reaction can be found in the severely atrophied tissue, the significance of the inflammation remained unclear because of the end-stage phase of the process (Freudiger 1971, Säteri 1975, Rimaila-Pärnänen and Westermarck 1982). There has been general consensus that the atrophy process is degenerative (Hill et al 1971, Säteri 1975, Hashimoto et al 1979, Rimaila-Pärnänen and Westermarck 1982). The degenerative nature was further supported by a study that followed the progression of acinar atrophy in a single dog (Westermarck et al 1993a). Westermarck et al (1993a) reported that the degenerative changes in acinar cells were, in the absence of marked inflammatory reaction, the most distinctive findings. In Study II, morphological changes in the pancreas were studied in a larger number of dogs. The severity of the morphological pancreatic changes was found to greatly depend on the site of the biopsy. Thus, failure to demonstrate inflammation by biopsy may be a result of the uneven distribution of the inflammatory changes in an affected pancreas.

Interpreting the histological findings in the subclinical phase of acinar atrophy raises the question of whether the inflammatory reaction is the initial abnormality or only secondary to pre-existing changes in the ultrastructure (Westermarck et al 1993a) or whether it is a result of functional abnormalities of the acinar cells. However, ultrastructural findings in Study II suggested that inflammatory reaction had an important role in the tissue destruction. Numerous intra-acinar lymphocytes were detected not only in partially affected acini, but also in unaffected acini, which revealed no other obvious ultrastructural changes.

In the pancreatic sections of dogs with partial PAA degenerative changes were also found (Study II). The degenerative changes of acinar cells observed were similar to those described previously by Westermarck et al (1993a) in one dog. These were characterized by a dilatation of RER, a swelling of mitochondria, and nuclear changes, which resulted in cell necrosis. However, these progressive degenerative changes are typically seen in cell swelling, a process that can be caused by various insults (Ghadially 1988). As the most obvious alterations in acinar cells in dogs with partial PAA occurred in association with inflammation, it is possible that inflammatory reaction is, at least partly, the cause of these degenerative changes. The

importance of the control dogs was obvious when the ultrastructural degenerative changes were interpreted. A mild dilatation of RER and mitochondrial swelling or inclusions were also occasionally observed in the control dogs. As a sensible organ, the pancreas is susceptible to artefactual changes induced by tissue handling (Jubb 1993) and thus some of the degenerative and even necrotic changes should be interpreted carefully. Westermarck et al (1993a) suggested that degenerative changes of acinar cells and zymogen granule fusion were among the first alterations. In Study II, no evidence of fusion or other abnormalities of zymogen granules were observed. On the contrary, well-preserved zymogen granules were found even in severely affected cells. The larger zymogen granules were considered to be prozymogen granules and could be found in both control dogs and dogs with partial PAA, indicating an active secretory stage of the normal acini (Tardini et al 1971, Motta et al 1997).

Interestingly, the morphological study (Study II) showed that in the subclinical phase of the atrophy process there is both necrotic and apoptotic acinar cell death. It is reported that apoptotic cell death may have a significant role in some immune-mediated disease (Patel and Gores 1995, Hammond et al 1997), as well as in natural and experimental pancreatic diseases (Oates et al 1986, Walker 1987,Walker et al 1993, Iovanna 1996). Recent studies using in situ hybridization to detect apoptosis indicate that apoptosis may be found to some extent in end-stage PAA (Steiner et al 2001). Based on a morphological study, it was difficult to assess the significance of apoptotic cell death in tissue destruction in the subclinical phase of PAA (Study II). The apoptotic death of acinar cells can occur in the normal pancreas of healthy animals and the frequency of apoptotic cell death may be underestimated because apoptosis occurs usually in scattered cells (Walker 1987, Patel and Gores 1995, Steiner et al 2001, Study II). Therefore, further studies are needed to estimate the role of apoptosis during the progress of tissue destruction in PAA.

9.3 The role of cellular and humoral immune responses in the pathogenesis of PAA

To study the possible role of cellular immune mechanisms in the pathogenesis of PAA, the tissue infiltrating lymphocytes were subjected to immunophenotyping (Study II and III). The results of the immunohistochemical study support the hypothesis that cell-mediated cytotoxicity plays a major role in the pathogenesis. At the onset of the acinar cell destruction in the subclinical phase, the majority of the lymphocytes infiltrating the tissue were T-lymphocytes and the intra-acinar lymphocytes were all T-cells (Study II). Further immunophenotyping of the T-cells showed that, in general, almost equal numbers of the CD4+ T-helper cells and CD8+ cells, representing mainly cytotoxic T-cells, were found. Typically, cytotoxic T-cells had infiltrated both the affected and normal acinar parenchyma**.** When a gradual destruction of acinar parenchyma was present, the cytotoxic T-cells were predominant (Study III).

In general, the findings in Studies II and III are comparable to those reported in cell-mediated autoimmune diseases, such as insulin-dependent diabetes mellitus and Hashimoto's thyroiditis. These disorders are characterized by a marked T-cell infiltration with a predominance of CD8+ cytotoxic cells at the onset of tissue destruction (Bottazzo et al 1985, Misaki et al 1985, Itoh et al 1993). However, it has been shown that macrophages and CD4+ T-helper cells may have a more significant role in the early phase of cell destruction in the cell-mediated diseases (Roep and De Vries 1992, Reddy et al 1995). Macrophages process and present antigens to CD4+ helper cells, which in turn are necessary to initiate both cell-mediated and humoral immune responses (Roep and De Vries 1992, Reddy et al 1995). Although the results of Study III suggest a significant role for the infiltrating cytotoxic CD8+ cells, it was not possible to assess the role of the different mononuclear cells in the early lesions. Macrophages were occasionally present among the infiltrating lymphocytes and in the areas of advanced atrophy. However, no infiltrating monocytes/macrophages were detected in the normal parenchyma.

In the subclinical phase, the presence of lymphoid follicles and an increased number of B-lymphocytes and plasma cells suggest that also humoral immune responses are activated when the destruction of acinar parenchyma is in progress (Study III). The intensity of the

inflammatory reaction was found to decrease on the development of total acinar atrophy. In the atrophied pancreas, scattered T-lymphocytes could be detected and only a few B-lymphocytes and plasma cells. The likely reason for the results is that any possible immune response which may have been present in the earlier phase was no longer active during the late clinical phase.

In Study III, the methods for screening the humoral immune status included serum protein electrophoresis, quantification of serum Ig-classes or subclasses and the assessment of serum autoantibodies. Polyclonal gammopathy, due to activation of the immune response, polyclonal B-cell stimulation and an increase in serum IgG and IgM, may be considered to be a typical finding in autoimmune diseases. Study III showed an increase in the serum gamma globulin fraction in the subclinical and clinical phases of the disease, but no significant changes in serum IgG and IgM levels were observed.

The activation of humoral immune responses can be further studied by identifying antibodies against certain autoantigens. Many autoimmune diseases have typical patterns of autoantibody production (Sinha et al 1990, Janeway et al 1999). Some of the autoantibodies can cause clinical manifestations, but in most cases they are used as predictors of the clinical course, to diagnose the disease or to assess its severity. Serum autoantibodies can be found in organ-specific autoimmune diseases despite the fact that they are considered to be mediated mostly by cytotoxic T-cells. The role of the autoantibodies in pathogenesis is usually questionable, but altogether, they provide further evidence of the autoimmune nature of the disease (Rose 1989, Sinha et al 1990, Roep and De Vries 1992, Roep 1996, Janeway et al 1999). Study III showed that in dogs with subclinical or clinical phase of PAA, serum antibodies directed against a coarse granular component of the pancreatic acinar cell cytoplasm were occasionally found. Similar antipancreatic antibodies have been reported in human exocrine pancreatic diseases (Lendrum and Walker 1975, Lankisch et al 1981, Rumenssen et al 1985). The granular immunofluorescence pattern indicated that the autoantibodies could be directed against acinar cell zymogen granules.

Although pancreatic-antibodies were not identified in the control dogs, the intensity of the positive reaction was so weak that the diagnostic usefulness of the antibodies is questionable with current methods (Study III). The antibodies can be a result of the autoimmune reaction, but for an unknown reason the reaction could not be detected in all the diseased dogs. On the

other hand, the antibodies can develop secondarily to tissue destruction and may thus not be pathognomonic to the disease. Because of the patchy nature of the destruction process in the pancreas, no effort was made to study the correlation between the immunohistological findings and the presence of autoantibodies.

It is possible that the methods used in the Study III were not sensitive enough to detect all possible antibodies. In addition to the indirect immunofluoresence test, the immunoblotting technique was used as a supportive method. Analysis of the immunoreactive bands failed to show any positive correlations to the subclinical or clinical phase of the disease. Analysis of the immunoblotting results, however, requires careful consideration as unwanted or non-specific staining reactions cause problems in interpretation. Immunoblotting has been previously used to study antipancreatic antibodies in dogs with clinical EPI, but because of the different method and conditions used a comparison of the previous and Study III is difficult (Simpson and Cobb 1998). The results of these studies were, however, similar.

The results of the immunological studies suggest that tissue destruction during progression of acinar atrophy is largely T-cell mediated, although the presence of numerous B-lymphocytes and pancreas-specific antibodies in sera of some dogs indicates that also humoral mechanisms are involved.

9.4 Response to long-term enzyme replacement treatment

Once the destruction of acinar tissue has become sufficiently advanced, inadequate production of digestive enzymes leads to clinical maldigestion signs of EPI. The primary treatment for EPI is to supplement each meal with pancreatic enzymes. Enzyme treatment is, however, lifelong and expensive. When EPI is diagnosed, owners are often anxious about the future quality of their dog's life and about the possible problems to be expected during long-term treatment. In Study IV, a survey was conducted to find answers to these questions. A comparison of the general health status and clinical signs was made between German shepherd dogs and rough-coated Collies treated for EPI and apparently healthy dogs of the same breeds. In these two breeds the clinical signs of EPI are most commonly due to end-stage PAA.

In general, the response to long-term enzyme replacement treatment was found to be good (Study IV). Basically, the treatment regimens were simple and consisted of adding a nonenteric-coated enzyme supplement to ordinary dog food. During long-term treatment, a marked resolution was achieved for most of the gastrointestinal tract signs typical of EPI, although it was not always possible to eliminate all signs. Good resolution was found especially for more serious signs, such as continous diarrhoea and malnutrition. The owners' assessments of the response to treatment were surprisingly positive. One reason for this finding may be that the clinical signs that often persisted; high faecal volume, yellow and pulpy faeces, and flatulence, were considered mild and, as time went on, they were accepted as part of normal life. Secondly, the study may lack information on dogs having a poor response to treatment, because at least some dogs were already euthanized during early treatment.

Despite basically similar treatment regimens, the responses to enzyme supplements in dogs with EPI varied considerably. Signs considered typical for EPI were almost completely controlled in half of the dogs and their general health was similar to that of clinically normal dogs. A poor response to treatment was found in 20% of dogs that had several signs typical for EPI. Study IV did not provide answers as to why some of the clinical signs were incompletely controlled and why the responses to treatment varied. Further studies are needed to investigate the reason for poor responses in individual dogs. Study IV indicated that the response to treatment was similar in German shepherd dogs and rough-coated Collies, and that the response was not affected by the type of nonenteric-coated enzymes fed, or by the length of treatment.

Nonenteric-coated enzyme formulations have been shown to be the most effective formulations in the treatment of EPI. The highest enzyme activity in the duodenum is achieved by feeding raw chopped pancreas, followed by powdered enzyme extracts (Westermarck 1987). In Finland, raw pancreas and powdered enzyme supplements are almost exclusively used. Thus, Study IV provided an opportunity to perform a thorough clinical comparison of the properties of these two supplements. Feeding raw chopped pancreas and powdered enzymes proved to be equally effective in controlling clinical signs. The choice of the two preparations was based more on the practical properties, availability and costs of the supplements. In some dogs, when the clinical signs were persistent with one supplement, changing to the other enzyme extract resulted in improved response to treatment. This

observation should be taken into account if the dog does not respond properly to first-choice enzyme supplement. In those countries where the use of raw pig's pancreas is not allowed, other animal sources of pancreas, may be used instead. Study IV showed that some of the dogs were successfully treated with pancreas from cattle, lamb or reindeer.

It has been reported that after an initial clinical improvement, decreasing the amount of enzymes fed did not give rise to a recurrence of the clinical signs (Westermarck et al 1995, Williams 2000). No actual effort was made to find the minimum effective dose for most dogs included in Study IV. The initial amount of enzyme fed was maintained during long-term treatment and was usually 3 g of powdered and 50 g to 100 g of raw chopped pancreas / meal for dogs weighing 20 to 35 kg. Although enzyme treatment is usually considered to be lifelong, especially in dogs with PAA, it has been shown that dogs initially showing severe clinical signs and responding to enzyme supplementation did not need continuous treatment. This indicates that, despite atrophy, the pancreas of these exceptional dogs may have some reserve secretory capacity remaining (Westermarck and Rimaila-Pärnänen 1989b). In the case of poor response to treatment, attempts have been made to increase the amount of enzymes fed. However, the value of increasing the dose remains uncertain and is probably of limited value (Hall et al 1991, Study IV).

The role of dietary modification in the treatment of EPI has remained questionable. This is partly because dietary changes are made when enzyme treatment is started and partly because of the great variations among the diets fed. Based on clinical studies, it may be concluded that special diets, although they could alleviate some clinical signs, are not usually necessary for long-term treatment (Westermarck et al 1990, Hall et al 1991, Westermarck et al 1995). Study IV supports this opinion. Dogs treated for EPI can be fed various kinds of ordinary dog food if particular attention is paid to individual needs and radical dietary changes are avoided. Dogs with EPI appear to be increasingly sensitive to sudden dietary changes and often have adverse reactions to various diets.

Of the supportive treatments recommended in the literature, antibiotics are most commonly used both in initial and long-term treatment (Hall et al 1991, Williams 2000). Study IV showed that during long-term treatment half of the dogs were receiving antibiotics when they experienced a recurrence of signs, such as diarrhoea, flatulence and borborygmus. Small intestinal bacterial overgrowth may be a problem also during enzyme treatment, thus partly

explaining the response to antibiotics in long-term treatment (Williams et al 1987, Westermarck et al 1993b). H_2- receptor antagonists are not routinely used in the treatment of EPI, but were found to be helpful for some dogs with clinical signs despite enzyme treatment (Study IV). Cobalamin deficiency is commonly reported in dogs with EPI. Thus, parenteral cobalamin injections are recommended when the serum cobalamin levels are low (Batt and Morgan 1982, Batt 1990, Hall et al 1991, Williams 2000). During long-term treatment, the indications for cobalamin treatment were found to be poor general health or a poor coat (Study IV). Dermatological signs were common especially in German shepherd dogs. Thus, there may be a need for other additional supportive treatments to control the dermatological signs (Study IV).

In conclusion, because the treatment of EPI is lifelong and usually expensive, treatment regimens should remain simple, but still pay particular attention to individual needs (Study IV). Treatment is started with nonenteric-coated enzyme formulations at the maintenance dose and continue feeding the dog its ordinary food. Because of the strong possibility of small intestinal bacterial overgrowth antibiotics may be valuable to hasten clinical improvement during the initial period of treatment. Parenteral cobalamin is recommended when low serum cobalamin values are detected. If the response to initial treatment turns out to be unsatisfactory, more aggressive and long-lasting supportive treatments are used. These include dietary modifications, antibiotics, and repeated cobalamin treatments. Any co-existing small intestinal disease should be diagnosed and treated. Nevertheless, it seems reasonable to assume that the response to treatment achieved during the first months will remain fairly stable during long-term treatment (Study IV). Even if short relapses of clinical signs may develop, permanent deterioration of the clinical condition in dogs with EPI during long-term treatment is probably uncommon. Thus, the prognosis for long-term enzyme replacement treatment is generally considered to be good.

10. CONCLUSIONS

I Studies of the early diagnosis and etiopathogenesis of PAA led to the following conclusions.

1. In German shepherd dogs and rough-coated Collies, PAA can be diagnosed in the subclinical phase before the total destruction of acinar tissue. Serum cTLI was found to be the most valuable diagnostic test for subnormal pancreatic function. Repeatedly low serum cTLI values (< 5.0 µg/L) in a clinically healthy dog were shown to be a sensitive marker of subclinical EPI and highly suggestive for partial PAA. To increase the accurancy of the diagnosis of the subclinical disease, repeated measurements (at least two consecutive samples) are needed, especially when a subnormal cTLI value (2.5 - 5.0 µg/L) is detected.

2. Histological findings during the progression of PAA were characteristic for an autoimmune disease showing marked lymphocytic infiltration into partially atrophied acinar parenchyma. The inflammatory reaction was most extensive in the border zone areas of normal and affected parenchyma and lymphocytes had infiltrated into apparently normal acinar tissue. The gradual destruction of acinar tissue was associated with inflammation. In the areas of more advanced tissue destruction, the findings were similar to those of end-stage PAA. Term “atrophic lymphocytic pancreatitis” was preferred to describe pathological findings.

3. Immunological studies suggested that both cellular and humoral immune responses have a role in the pathogenesis of acinar atrophy, although tissue destruction seems to be largely mediated by cellular immune mechanisms. Immunohistochemical analysis showed that in the subclinical phase, the majority of the infiltrating lymphocytes were T-cells, with an almost equal number of CD4+ T-helper and CD8+ cytotoxic T-lymphocytes. Cytotoxic T-cells were predominant in sections where the gradual destruction of acinar parenchyma was present. The occurence of serum autoantibodies against pancreatic acinar cells not only suggested activation of the humoral immune response but also further indicated the autoimmune nature of the disease.

Thus, the etiopathogenetic study suggests that PAA in German shepherd dogs and rough-coated Collies is a consequence of autoimmune-mediated atrophic lymphocytic pancreatitis. Both the previous information about the inheritance of PAA and the current findings of marked infiltrative T-lymphocyte inflammation in the gradually atrophying acinar parenchyma suggest that the disease is of an autoimmune nature. The major role of cell-mediated cytotoxicity in tissue destruction was shown.

II The study of the long-term response to enzyme replacement treatment showed that gastrointestinal tract signs considered typical for dogs with EPI were kept almost completely in control with nonenteric-coated enzyme supplements in half of the dogs. The general health of these dogs was similar to that of clinically normal dogs of the same breeds. Despite basically similar treatment regimens, the responses varied considerably. Poor response to treatment was observed in 20% of the dogs with EPI. Although dietary sensitivities were common, the need for dietary treatment was minimal. Short relapses of clinical signs may develop during long-term treatment. To control these signs, antibiotics were administered to half of the dogs during treatment. Response to treatment achieved during the first months of treatment usually remained fairly stable during long-term treatment and the permanent deterioration of the clinical condition in dogs with EPI during long-term treatment is probably uncommon. Thus, in general, the prognosis for the long-term treatment of EPI with noneneteric-coated enzyme supplements is considered to be good.

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